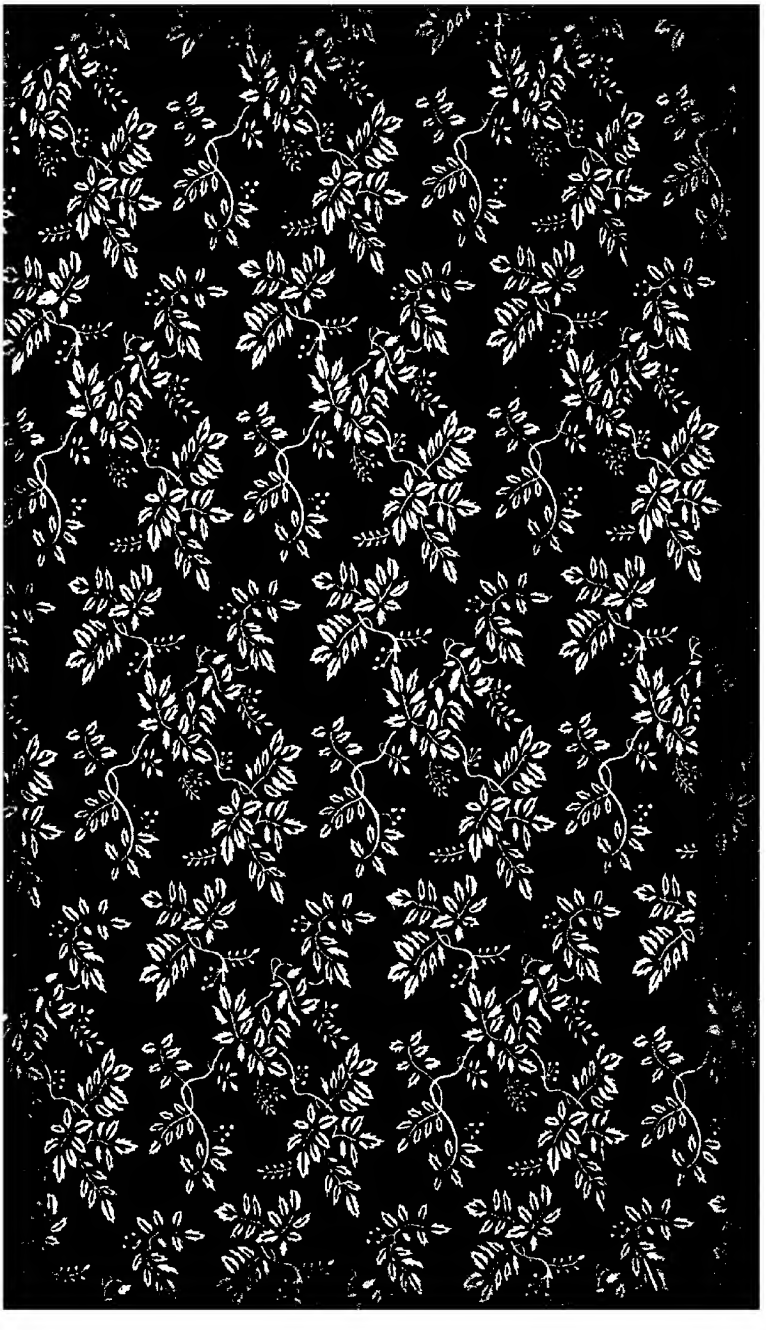
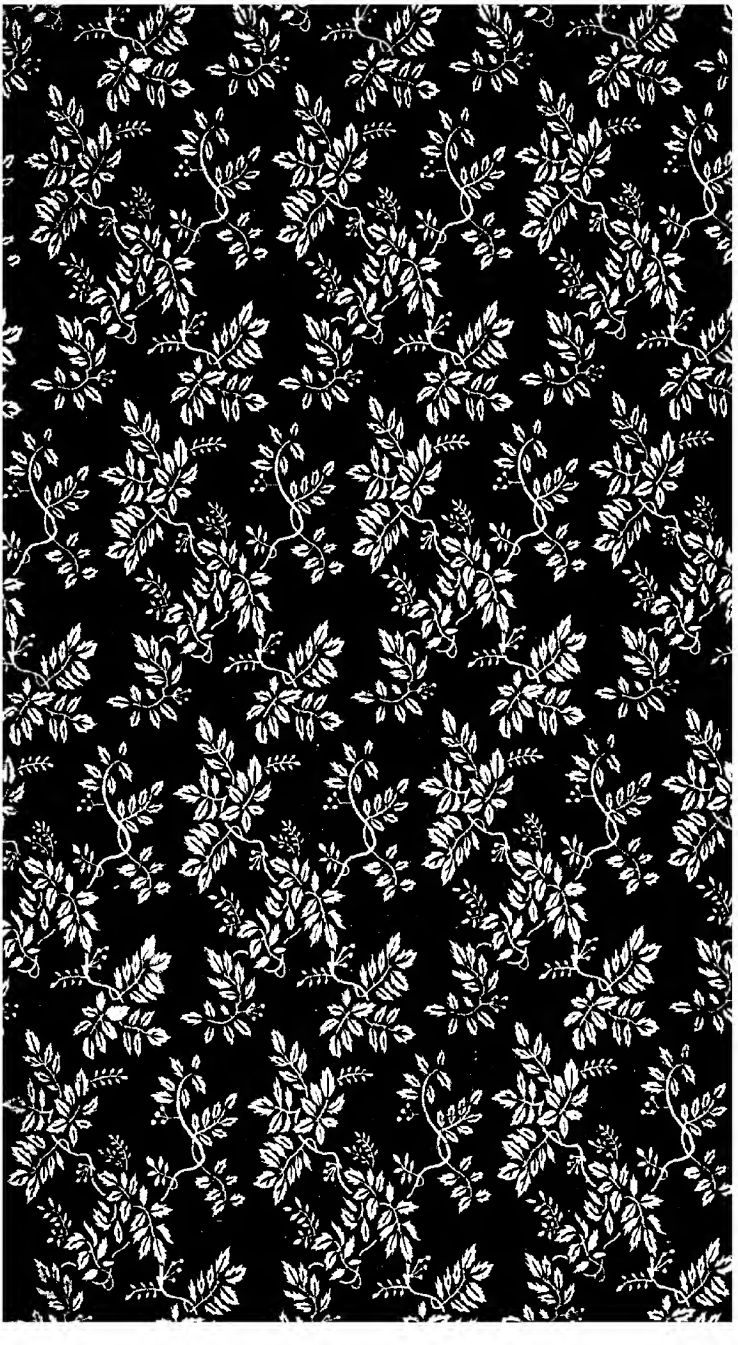




THE JOURNAL OF AGRICULTURAL SCIENCE

Volume III. 1908—10

CAMBRIDGE:
at the University Press

LONDON: Fetter Lane, C. F. CLAY, Manager
also H. K. LEWIS, Gower Street
and WILLIAM WESLEY & SON, 28, Essex Street, Strand
EDINBURGH: 100, Princes Street
BERLIN: A. Asher and Co.
LEIPSIC: F. A. Brockhaus
NEW YORK: G. P. Putnam's Sons
BOMBAY AND CALCUTTA: Macmillan and Co., Ltd.
Entered at the New York Post Office as Second Class Matter

Cambridge:
PRINTED BY JOHN CLAY, M.A.
AT THE UNIVERSITY PRESS.

CONTENTS.

Part 1 (December, 1908).

Part 2 (October, 1909).

Part 3 (September, 1910).

Part 4 (December, 1910).

VOLUME III DECEMBER, 1908 PART I

THE RATE OF FERMENTATION OF CIDERS AND PERRIES.

BY

B. T. P. BARKER, M.A.,

National Fruit and Cider Institute.

THE most striking feature of the fermentation of ciders and perries is the great variation in the rate of fermentation which is met with. It is the regular experience of cider makers that casks of cider, standing side by side in the same cellar, made at the same time, and treated throughout in similar fashion, ferment in nearly every instance with different degrees of rapidity. For example, the specific gravity in one cask may fall from 1·050 to 1·046 in the course of three or four weeks, while in its neighbour the drop may be from 1·050 to 1·000. Variations of this kind make it difficult to exercise much control over the nature of the product, and absolute uniformity in the character of the mature ciders cannot be expected. In point of fact uniformity in every case is not desired, since ciders of various grades of sweetness and dryness are required for market purposes. In other words, ciders with specific gravities ranging from 1·000 to 1·040 or even higher, all find a place on the market. It is not satisfactory, however, that the production of a cider of any particular type should be left mainly to chance, as is generally the case: and to obtain control, the power of producing at will a cider of low or of high specific gravity is essential, and involves a knowledge of the factors which determine and govern the rate of fermentation. The work described in this paper was undertaken with the object of gaining some idea of the nature and influence of these factors, and of ascertaining how they could be utilised to practical advantage. It was carried out at the National Fruit and Cider Institute, where the ciders investigated were made, in some cases on a practical scale in the cider house, and in the remainder on a more limited scale in the laboratory.

The relation between the variety of apple used and the rate of fermentation.

The Tables A and B (pp. 4, 5, 6) give records of the drop in specific gravity of ciders and perries investigated during the seasons 1904—05, 05—06, and 06—07. They were made in each case in the cider house, and were kept during the course of fermentation in the cellar, at the ordinary cellar temperature, which, although varying according to the outdoor temperature, usually ranged between 45° and 50° F. The total bulk of the juice was usually about 54 gallons, but in certain cases the quantity was as small as 19 gallons, and in others as much as 120 gallons. Each cider or perry was made from one kind of apple or pear only, the fruit being milled when it was judged to be in fit condition. The general treatment was the same in all cases referred to in Table A, the juice after its expression from the pulped fruit being "keeved" and afterwards pumped into the fermenting cask, which was filled to the bung-hole, and allowed to remain there undisturbed until the time of filtration. The weekly records are not extended beyond that point, since the process of filtration causes a radical alteration in the normal course of fermentation. The records were for the most part taken at regular weekly intervals, but in those cases where the interval was longer, the total drop during such periods has been averaged so as to show approximately the weekly fall for the intervening time.

Table A gives the weekly records of gravities taken direct from the liquor in cask. Table B gives the daily records of gravities of the ciders kept at 25° C. In these cases sample bottles of the fresh juices were taken from each cask as soon as filled, and the gravities given are those of the liquids in these bottles, the same ciders in bulk being kept in casks in the cellar at the ordinary temperature. There was very little difference between the comparative rates of fermentation of the different ciders in bottle at 25° C., and in cask at the ordinary temperature.

It will be seen from these tables that there are wide variations in the rate of fermentation of the ciders made from different varieties of apples: and that in some cases extreme variations occur in the ciders made from the same variety. As an instance of this, the two Strawberry Norman ciders made in 1904—5 may be referred to. But while it is not uncommon to find such variations in the case of any particular variety, on the whole it may be claimed that there is some relation between the rate of fermentation of the cider and the variety

of apple from which the cider is made. The Kingston Black ciders and Oldfield perries in each season, referred to in the tables, fermented at a moderately slow rate. The Sweet Alford, Cap of Liberty, and Frederick, among others, also regularly fermented more slowly than the average cider. No good examples of ciders with consistently rapid fermentation can be quoted from the above tables with the exception of Northwood, since such varieties are noted for the poor quality of the product, and were therefore not procured in quantity for experimental purposes in successive seasons. However, in several cases small lots of the fruit were tested in the laboratory, and the results in those experiments proved that the rate of fermentation is normally rapid for many varieties. Such kinds are Morgan Sweet, Fair Maid of Devon, Tom Putt, and Broadleaf, which also on the single occasion tested in the cider house and referred to in the tables (pp. 4, 5, 6), gave similar results. The experience of practical cider makers confirms the results obtained for the varieties mentioned as yielding slowly or rapidly fermenting ciders.

Although a certain amount of agreement in the results for these varieties has been noted, which is sufficient perhaps to justify the statement that there is a relation between the variety of apple or pear and the rate of fermentation of the juice, it is highly probable that this relation is much closer than appears from the statistics given. There are other factors which have a material influence upon the rate of fermentation, and they undoubtedly tend to obscure the "variety" influence. They are considered in detail below; and among those likely to be largely accountable for the different behaviour of ciders made from the same variety of apple may be mentioned the condition of ripeness of the fruit at the time of milling, the extent to which it has been exposed to the sun, and the kind of soil upon which it was grown.

The causes of the variations in the rate of fermentation.

While it has thus been shown that there is to a certain extent some connexion between the rate of fermentation and the variety of apple or pear from which the cider or perry is made, the more immediate causes of the variations remain to be considered. The various factors which were investigated include the chemical composition of the juice, the state of ripeness of the fruit at the time of making, the organisms taking part in the fermentation, the temperature at which the fermentation was conducted, and the aeration of the cider. The nature of the

TABLE A.

Name of Variety	Specific Gravity											
	Fresh Juice	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week	9th week	10th week	11th week
Apples 1904—5												
Broadleaf	1·055	1·050	1·045	1·039	1·033	1·022	1·010	—	—	—	—	—
Broad Styre	1·053	1·051	1·049	1·047	—	—	—	—	—	—	—	—
Brown Thorn	1·049	1·047	1·045	1·043	1·041	1·038	1·035	1·031	1·027	1·023	—	—
Cap of Liberty	1·062	1·060	1·057	1·054	1·050	1·046	1·042	1·039	1·037	1·036	1·035	1·034
Cherry Norman	1·052	1·050	1·048	1·045	1·042	1·039	1·036	1·034	1·032	1·030	1·029	1·028
Dabinet	1·055	1·055	1·054	1·053	1·052	1·050	1·048	1·046	1·044	1·042	1·040	1·038
Early Red Jersey	1·049	1·049	1·048	1·046	1·043	1·040	1·037	1·034	1·032	1·030	1·028	1·026
Fair Maid of Devon	1·041	1·038	1·034	1·029	1·024	1·019	1·015	1·011	1·007	—	—	—
Frederick	1·050	1·050	1·050	1·048	1·045	1·042	1·039	1·036	1·034	1·033	1·032	1·031
Gloucester French	1·057	1·053	1·049	1·045	1·041	1·035	1·030	1·026	—	—	—	—
Kingston Black I.	1·058	1·057	1·056	1·051	1·046	1·042	1·038	1·035	1·032	1·029	1·026	1·023
,, ,, II.	1·057	1·057	1·056	1·053	1·050	1·047	1·044	1·042	1·040	1·038	1·036	1·034
Knotted Kernel	1·054	1·054	1·054	1·051	1·047	1·043	1·039	1·036	1·034	1·033	1·032	1·031
Master's Jersey	1·065	1·065	1·063	1·061	1·058	1·055	1·052	1·049	1·046	1·044	1·042	1·040
Morgan Sweet	1·045	1·043	1·040	1·028	1·015	1·009	1·005	1·002	—	—	—	—
Northwood	1·046	1·044	1·040	1·034	1·030	1·027	1·024	1·021	—	—	—	—
Pytheres	1·056	1·054	1·051	1·045	1·039	1·033	1·026	—	—	—	—	—
Red Foxwhelp	1·042	1·039	1·036	1·034	1·032	1·030	1·027	1·025	1·023	1·021	—	—
Red Soldier	1·058	1·056	1·054	1·050	1·045	1·041	1·039	1·037	1·035	1·033	1·032	1·031
Royal Jersey	1·071	1·070	1·069	1·063	1·057	1·053	1·050	1·047	1·044	1·041	1·038	1·035
Strawberry Norman I.	1·060	1·056	1·050	1·041	1·031	1·025	1·020	1·015	1·010	—	—	—
,, ,, II.	1·048	1·047	1·045	1·042	1·040	1·037	1·035	1·033	1·031	1·029	1·027	1·025
Sweet Alford	1·049	1·048	1·047	1·044	1·040	1·038	1·036	1·034	1·032	1·031	1·030	1·029
White Norman I.	1·050	1·049	1·047	1·044	1·041	1·039	1·037	1·035	1·034	1·033	1·032	1·031
,, ,, II.	1·050	1·050	1·049	1·048	1·047	1·045	1·044	1·043	1·042	1·041	1·040	1·039
Woodbine	1·051	1·047	1·041	1·035	1·030	1·028	1·026	1·024	1·023	1·022	1·021	1·020
Worcester Red	1·047	1·046	1·042	1·037	1·031	1·026	1·021	1·015	—	—	—	—
Yarlington Mill	1·050	1·047	1·043	1·039	1·036	1·033	1·030	1·026	1·023	—	—	—

Name of Variety	Specific Gravity											
	Fresh Juice	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week	9th week	10th week	11th week
Pears 1904—5												
Butt	1·064	1·063	1·059	1·054	1·051	1·048	1·045	1·042	1·039	1·036	1·033	1·031
Oldfield I.	1·047	1·047	1·046	1·045	1·044	1·042	1·040	1·040	1·038	1·037	1·036	1·035
,, II.	1·067	1·067	1·067	1·066	1·064	1·062	1·060	1·058	1·057	1·056	1·055	1·054
Red Longland	1·046	1·042	1·036	1·030	1·025	1·020	1·015	1·011	1·007	—	—	—
Apples 1905—6												
Ashton White	1·055	—	—	—	—	—	—	—	1·038	1·038	1·036	1·034
Belle Norman	1·049	—	—	—	1·038	1·037	1·034	1·032	1·029	1·027	1·025	1·025
Cap of Liberty	1·059	1·059	1·056	1·052	1·045	1·040	1·037	1·034	—	—	—	—
Chiswell George	1·048	1·047	1·044	1·040	1·037	1·033	1·028	1·024	1·020	1·017	1·014	1·011
Cluster	1·051	1·051	1·050	1·044	1·035	1·029	1·025	1·021	1·017	1·014	1·013	—
Cummy Norman I.	1·058	1·056	1·048	1·036	1·026	1·018	—	—	—	—	—	—
,, ,, II.	1·064	1·054	1·039	1·027	1·016	1·007	—	—	—	—	—	—
Farmer's Glory	1·048	1·044	1·038	1·032	1·025	1·018	1·010	1·002	—	—	—	—
Frederick	1·050	1·048	1·044	1·037	1·034	1·029	1·023	1·020	1·018	—	—	—
Horners	1·061	1·057	1·052	1·047	1·041	1·039	1·032	1·027	1·023	1·021	1·017	1·014
Kingston Black	1·064	1·063	1·061	1·056	1·052	1·047	1·043	1·039	1·037	1·035	1·035	—
Northwood	1·049	1·045	1·037	1·026	1·019	1·013	1·011	1·005	—	—	—	—
Red Jersey	1·064	1·060	1·053	1·041	1·034	1·027	1·022	1·019	1·013	—	—	—
Ridgeway	1·046	1·046	1·038	1·036	1·027	1·021	1·018	1·015	1·010	—	—	—
Sweet Alford	1·051	1·050	1·047	1·043	1·040	1·035	1·031	1·027	1·025	1·024	1·021	1·020
White Jersey	1·051	1·044	1·034	1·027	1·020	1·014	1·011	1·008	1·006	—	—	—
White Norman	1·059	1·055	1·050	1·044	1·035	1·030	1·025	1·022	1·020	1·017	1·015	—
Woodbine	1·055	1·048	1·042	1·036	1·032	1·032	1·029	1·025	1·021	1·019	—	—
Yeovil Sour	1·052	1·051	1·042	1·035	1·033	1·029	1·026	1·024	—	—	—	—
Pears 1905—6												
Oldfield I.	1·060	1·054	1·054	1·050	1·045	1·040	1·035	1·032	1·029	1·025	—	—
,, II.	1·054	1·053	1·051	1·049	1·047	1·041	1·033	1·029	1·024	1·022	1·019	1·017
,, III.	1·069	1·069	1·069	1·061	1·055	1·048	1·043	1·041	1·037	1·034	1·034	—

TABLE B.

Name of Variety	Specific Gravity										
	Fresh juice	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day	11th day
Apples 1906—7												
Ansell	1·056	1·055	1·053	1·039	1·028	1·020	1·012	1·007	1·003	—	—	—
Cap of Liberty	1·063	1·062	1·055	1·049	1·043	1·038	1·030	1·024	1·022	1·020	1·017	1·014
Cherry Norman	1·055	1·050	1·044	1·040	1·036	1·034	1·029	1·023	1·022	1·020	1·018	—
Cluster Jersey	1·052	1·046	1·041	1·035	1·028	1·022	1·020	1·016	1·012	—	—	—
Counsellors	1·071	1·068	1·062	1·041	1·020	1·009	1·001	—	—	—	—	—
Cowarne Red	1·054	1·051	1·046	1·044	1·037	1·034	1·029	1·027	1·026	1·024	1·020	—
Dabinet	1·052	1·047	1·043	1·036	1·029	1·022	1·017	1·013	—	—	—	—
Davis' Favourite	1·042	1·042	1·036	1·032	1·027	1·023	—	—	—	—	—	—
Frederick	1·055	1·049	1·045	1·041	1·036	1·033	1·030	1·028	1·025	1·025	1·024	1·022
Horners	1·053	1·051	1·048	1·047	1·046	1·044	1·041	1·039	1·037	1·037	1·035	—
Kingston Black I.	1·061	1·057	1·055	1·049	1·047	1·043	1·039	1·037	1·035	1·033	1·032	1·032
,, ,, II.	1·073	1·071	1·065	1·051	1·044	1·035	1·028	1·024	1·021	1·020	1·017	1·015
,, ,, III.	1·070	1·068	1·063	1·055	1·051	1·045	1·041	1·037	1·035	1·032	1·031	1·031
Langworthy	1·051	1·049	1·047	1·045	1·044	1·041	1·040	1·038	1·038	1·037	1·036	1·035
Loram's Sweet White	1·069	1·067	1·057	1·049	1·041	1·032	1·025	1·022	1·020	1·019	1·017	1·015
Major	1·060	1·056	1·052	1·050	1·048	1·046	1·043	1·039	1·039	—	—	—
Master's Jersey	1·062	1·055	1·050	1·043	1·036	1·032	1·029	—	—	—	—	—
Northwood	1·060	1·057	1·049	1·041	1·030	1·023	1·015	1·011	1·006	—	—	—
Pople's Gutter	1·047	1·044	1·040	1·033	1·027	1·024	1·021	1·019	—	—	—	—
Prince Albert	1·070	1·061	1·055	1·044	1·035	1·029	1·026	1·022	1·019	1·019	1·018	1·017
Pytheres	1·051	1·049	1·042	1·036	1·027	1·023	1·015	1·011	1·008	—	—	—
Royal Wilding	1·057	1·053	1·051	1·043	1·034	1·025	1·019	1·014	1·010	—	—	—
Skyrme's Kernel	1·049	1·047	1·043	1·041	1·036	1·034	1·032	1·031	1·030	1·028	1·026	1·025
Strawberry Norman	1·056	1·051	1·050	1·044	1·039	1·034	1·029	1·025	1·023	1·021	—	—
Sweet Alford	1·059	1·057	1·053	1·053	1·052	1·049	1·046	1·046	1·044	1·042	1·039	1·037
Symes' Sweet	1·052	1·052	1·048	1·044	1·041	1·039	1·035	1·031	1·030	1·028	1·026	1·024
Tom Putt	1·052	1·049	1·035	1·021	1·012	1·003	1·000	—	—	—	—	—
White Norman	1·054	1·048	1·041	1·036	1·031	1·026	1·020	1·015	1·011	1·008	1·007	—
Yarlington Mill	1·053	1·050	1·047	1·044	1·037	1·035	1·033	1·031	1·029	1·027	1·025	1·024
Pears 1906—7												
Butt	1·053	1·047	1·042	1·035	1·027	1·025	1·022	1·020	1·018	1·017	1·016	1·015
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

soil upon which the fruit is grown may have an important influence: and it is commonly asserted by practical cider makers that they have proved it to be the case. The soil factor, however, must be left for future consideration, since definite conclusions based on the limited data at present available are not justified.

The influence of the chemical composition of the juice.

The chemical composition of the juice obtained from the different kinds of apples varies considerably. Analyses of French and German cider apples have been published by Truelle (7) and Kulisch (5) among others. Allwood (1) has given details of the composition of the juice of several American varieties, while Hogg and Graves Bull (4), Lloyd (6) and myself (2) have published analyses of a very large number of English vintage varieties. They may be grouped into three main classes, "sours," "sweets," and "bittersweets," according to the composition of their juice. The "sours" include those with juices containing normally ·45 per cent. malic acid and upwards; the "sweets" those with juices containing normally less than ·45 per cent. malic acid and ·20 per cent. tannin; and the "bittersweets" those with juices containing normally less than ·45 per cent. malic acid and more than ·20 per cent. tannin. The percentage of sugar in the juice varies widely, even for the same variety. For vintage purposes the usual contents of the juice which are considered of primary importance are sugar, tannin and malic acid. The percentages of the other substances present in the juice, including mucilages, nitrogenous substances, and ash constituents, are very small and comparatively little attention has been paid to them.

The analyses of the ciders dealt with in Table A have been given elsewhere (2). A detailed comparison of the composition of the various fresh juices as regards total solid matter, sugar, tannin, malic acid, and total extractives, with their rates of fermentation, fails to show any signs of a direct relation between the quantity present of any one of those substances and the rate of fermentation. For example, Counsellor cider with an original gravity of 1·071 showed a drop to 1·001 in 6 days at 25° C., while Kingston Black cider with an original gravity of 1·070 only dropped to 1·045 in the same period. Again, Strawberry Norman cider containing ·650 per cent. of tannin showed a drop in specific gravity from 1·060 to 1·010 while Royal Jersey cider containing ·700 per cent. tannin in the same period only dropped from 1·071 to 1·044. Also, Fair Maid of Devon cider containing ·75 per cent. malic acid

showed a fall from 1·041 to 1·007, while Cap of Liberty cider containing 1·01 per cent. malic acid suffered in the same period a drop from 1·062 to 1·037 only. At the same time the amounts of these substances in some cases, especially in the case of tannin, may have some influence, although it is certainly overwhelmed and obscured by that of other factors.

Since the observed differences in the rate of fermentation of the juices of different varieties of apples and pears are not due to any of the substances just mentioned, it follows that either the chemical composition has little influence or else the effect is brought about by one or more of the substances which are present in very limited quantities and are not determined in the usual analyses. There were certain reasons for believing that the latter alternative was the correct one, since, as will be pointed out later, the kinds of yeast present did not appear to be responsible for the differences, and the nutrition of the yeasts seemed defective. It was previously known, too, that the fermentation of many fruit juices is slow and irregular owing to the lack of assimilable nitrogenous substances, required for satisfactory yeast nutrition. It seemed desirable, therefore, to ascertain if, in the slowly fermenting apple and pear juices, any of the elements necessary for the proper nutrition of the yeasts were absent or, if present, occurred in quantities too small to meet the full demands of the yeasts. The subject was not attacked by direct estimation of the essential elements, partly because the quantity required in some cases is so small, and partly because of the difficulty of determining if any particular element occurred in a form readily assimilable by the yeast. The test method adopted was to add to the cider small quantities of each of the respective elements required in forms known to be assimilable by the yeast, and to watch the effect on the rate of fermentation. Several different ciders were tested in this way, and in every case the rate of fermentation was only affected when assimilable nitrogenous substances were added. None of the other elements requisite for yeast nutrition had any appreciable effect in the fermentation. Tables C and D (p. 9) give the results obtained for Oldfield perry and White Norman cider. The following method was employed:

200 c.c. of the juice in an Erlenmeyer flask was used in each case, and the cider was left undisturbed at 25° C. during the course of the experiments. The experiments were started in February 1905, the juices thus being about three months old at the time, during which

time they had been subjected to fermentation in the ordinary manner in bulk in the cider house, the samples being taken direct from the casks for the purpose of these tests. The addition of one per cent. ammonium tartrate caused a rise in specific gravity of 5 points, and of ·5 per cent. potassium phosphate and magnesium sulphate respectively, 2 points; so that actually the specific gravities of the Oldfield *b*, *c*, *d* and *e* were 1·058, 1·054, 1·051 and 1·051 respectively at the start of the experiment as compared with 1·049 for the untreated juice *a*, and of the White Norman *b*, *c*, *d* and *e* 1·045, 1·041, 1·038 and 1·038 respectively as compared with 1·036 for *a*. In all cases a certain amount of acetification occurred, but there was less in *b* and *c* of both series—the only ciders which fermented at all actively—than in the others.

TABLE C. *Oldfield Perry.*

Specific gravity of untreated juice at the beginning of the experiment 1·049.				
,,	,,	after 20 days at 25° C.	in	*a* 1·045
,,	,,	,,	,,	*b* 1·014
,,	,,	,,	,,	*c* 1·006
,,	,,	,,	,,	*d* 1·048
,,	,,	,,	,,	*e* 1·051
,,	,,	,,	,,	*f* 1·049
,,	,,	,,	,,	*g* 1·047

TABLE D. *White Norman Cider.*

Specific gravity of untreated juice at the beginning of the experiment 1·036.				
,,	,,	after 14 days at 25° C.	in	*a* 1·030
,,	,,	,,	,,	*b* 1·011
,,	,,	,,	,,	*c* 1·011
,,	,,	,,	,,	*d* 1·032
,,	,,	,,	,,	*e* 1·032
,,	,,	,,	,,	*f* 1·030

In both series *a* was the juice without any addition

b was juice to which was added 1 per cent. ammonium tartrate
·5 ,, potassium phosphate
·5 ,, magnesium sulphate
·05 ,, calcium phosphate
and a trace of ferric chloride

c ,, ,, 1 per cent. ammonium tartrate only
d ,, ,, ·5 ,, potassium phosphate only
e ,, ,, ·5 ,, magnesium sulphate only
f ,, ,, ·05 ,, calcium phosphate only
g ,, ,, a trace of ferric chloride only.

The results show clearly that the addition of assimilable nitrogenous substances to slowly fermenting ciders is required to produce an

increase in the rate of fermentation. Indeed, their addition to any juice usually results in an increased rate. Consequently it must be concluded that apple and pear juices are naturally more or less deficient in those substances, and that the rate of fermentation depends on the quantity, and possibly the nature, of those present, other conditions being equal.

Ammonium tartrate was used in these experiments as the source of nitrogen for yeast assimilation, but in other cases the effects of peptone and asparagin were also tested. As with ammonium tartrate, the rate of fermentation was increased by their addition, but the increase was as a rule not so pronounced as with the former substance.

That the addition of assimilable nitrogenous material causes an increase in the rate of fermentation in fresh juices as well as in the older liquors was proved by the following example.

TABLE E.

Juice from mixed apples kept at 25° C.	Specific gravity				
	at beginning of experiment	at the end of 24 hrs.	do. 48 hrs.	do. 72 hrs.	do. 96 hrs.
Juice alone.....	1·038	1·036	1·029	1·021	1·016
Juice + ·1% asparagin ...	1·038	1·032	1·018	1·004	1·001

The effect of the addition of assimilable nitrogenous substances is so pronounced, that this factor of the quantity of such substances naturally present in the juice is quite sufficient in itself to account fully for the differences in the rate of fermentation of any ciders, made and fermented under similar conditions.

The chemical composition of apple and pear juice varies considerably according to the state of ripeness of the fruit from which the juice was obtained. The variation is naturally most marked in the cases of the contents of sugar and of the specific gravity, but the amounts of malic acid, tannin and many of the other constituents occurring in smaller quantity are also affected. It would be expected therefore that the rate of fermentation might be likewise affected. This was proved to be the case by comparing the records of the rate of fermentation of the juices obtained from fruit grown on the same tree but pressed at different stages of ripeness. A large quantity of Early Red Jersey

apples was gathered on the same day from one of the trees of this variety in the orchard at the Institute, before they were fully ripe. They were sorted into three lots, one lot composed entirely of green unripe specimens, another of well-coloured fruit more or less ripe and the third of fruit showing the first signs of decay due to over-ripeness. The juice was expressed from samples taken from each lot at weekly intervals and the rate of fermentation at the ordinary laboratory temperature (about 60° F.) noted in each case. The quantity of juice used in each instance was 10 ozs.; and this was kept in 10 oz. bottles, so that each bottle was filled to the neck and any possible influence due to the action of air on the juice thus rendered the same for all. Table F contains the records of the specific gravities taken after various intervals. The date on which the first specific gravity in each case is recorded is the date of pressing.

TABLE F.

Juice obtained from early Red Jersey apples	Specific gravity								
	Sept. 29	Oct. 6	Oct. 14	Oct. 21	Nov. 7	Nov. 21	Dec. 22	Jan. 12	Jan. 29
A 1 unripe...	1·047	1·045	1·035	1·026	1·012	1·006	·998	—	—
A 2 ripe.....	1·049	1·047	1·044	1·040	1·034	1·030	1·020	1·019	1·019
B 1 unripe...	—	1·050	1·048	1·040	1·023	1·011	·998	—	—
B 2 ripe.....	—	1·052	1·051	1·050	1·045	1·042	1·032	1·031	1·029
C 1 unripe...	—	—	1·0505	1·048	1·036	1·023	1·006	—	·999
C 2 ripe.....	—	—	1·051	1·050	1·047	1·044	1·039	1·037	1·035
C 3 over-ripe	—	—	1·045	1·045	1·044	1·041	1·035	1·033	1·031
D 1 unripe...	—	—	—	1·052	1·050	1·027	1·006	—	·998
D 2 ripe.....	—	—	—	1·055	1·055	1·049	1·037	1·032	1·025
D 3 over-ripe	—	—	—	1·056	1·055	1·046	1·029	1·023	1·018
E 1 unripe...	—	—	—	—	1·053	1·048	1·000	—	—
E 2 ripe	—	—	—	—	1·053	1·050	1·034	1·026	1·018
E 3 over-ripe	—	—	—	—	1·055	1·053	1·009	1·002	1·001

From this table it will be seen that in every case the juice obtained from unripe fruit fermented rapidly to dryness, and that there was no great difference in the rate in either instance. The slowest rate was in C 1; and it is significant that the slowest rates for the ripe and over-ripe juices occurred in C 2 and C 3, which were made on the same day as C 1. The ciders obtained from the ripe samples of fruit showed very interesting variations in the rate. Thus B 2 fermented more slowly than A 2, and C 2 rather more slowly than B 2: then the rate gradually increases in the later samples, the rate in D 2 being more

rapid than in C 2, and in E 2 than in D 2. In the latter respect the over-ripe samples C 3, D 3 and E 3 also agree. C 3 was the first of the over-ripe samples made, since before that time there was not sufficient distinction between that fruit and the fruit used for the "ripe" samples to justify any separation. Therefore the statistics for A 2 and B 2 may fairly stand for the over-ripe class also, in which case it will be noticed that there is a similar gradual decrease, and then an increase, in the comparative rates of fermentation of the samples made up in successive weeks for the over-ripe as well as for the ripe fruit. The same may be said for the unripe fruit; but on account of the fermentation being much more rapid the variation is not so well marked.

The terms "unripe," "ripe" and "over-ripe," as used above, need qualification. They are used in a comparative sense only, since obviously the absolute condition of ripeness varied from week to week. Thus the "ripe" specimens, for example, were in reality only properly and fully ripe between about October 4th and October 16th. Each set of fruit therefore during the course of the experiment actually passed through stages of comparative unripeness to full maturity and onwards to over-ripeness.

Allowing for this, it is clear therefore from the above statistics that, during the course of ripening of an apple, changes of chemical composition occur in the juice, which cause a gradual reduction in the potential rate of fermentation, until the apple attains full ripeness, after which point, as maturity merges into decay, the rate gradually increases.

An interesting point arising out of the preceding experiment, is the relation between the extent of development of colour in the fruit and the rate of fermentation. In that experiment, although the green unripe fruit was never at any given moment as ripe absolutely as the well-coloured ripe fruit, nevertheless towards the end of the period over which the experiment lasted, it was actually in a much riper state than the coloured ripe fruit in the early stages of the period. But, in spite of that, in no instance was the rate of fermentation of its juice as slow as that of the juice of the other in its most rapid example. Other experiments with well-coloured and poorly-coloured fruits of several other varieties of apples have been made; and invariably the juice from the well-coloured fruit ferments more slowly than that from the poorly-coloured fruit of the same variety. Since the extent of the development of the colour depends upon the degree to which the fruit is exposed to the sun, it follows that direct sunlight seems to play an

important part in influencing the chemical changes within the fruit, which affect the rate of fermentation of the juice.

From what has been stated above with regard to the part played by the assimilable nitrogenous substances in the juice in determining the rate of fermentation, it is to be expected that the differences in the rate at the various periods of ripening are due to variations in the quantities of these substances in the juice. Although this has not been directly proved by analysis, it is probably correct, since the addition of ammonium tartrate to juices from ripe fruit causes a decided increase in the rate of fermentation, thus showing that the comparatively slow rate is due, very considerably at least, to a deficiency of assimilable nitrogenous material.

Occasionally the mucilaginous substances in the juice are responsible indirectly for an appreciable effect on the rate of fermentation. They gradually undergo certain changes in the fermenting juice which frequently lead to the separation in insoluble form of various pectinous compounds. These are sometimes deposited gradually in small flocculent masses, but on other occasions, especially if the fruit has been at a certain stage of ripeness when milled, and if the acidity of the juice is below ·2 per cent. malic acid, they are thrown out of solution by a clotting action. A thin jelly-like clot is formed throughout the liquid which slowly contracts in the manner of other clots, retaining all the solid matter originally suspended in the juice and also practically all the yeast cells. The liquor is in this way rendered as clear as if it had been filtered. The enclosure of the yeast cells within the contracting clot thus in a sense removes them from the juice; and fermentation is more or less checked until the clot disintegrates, which at times does not occur until many weeks later.

The influence of the yeasts.

Although it has been demonstrated that the rate of fermentation depends primarily upon the amount of nitrogenous yeast food present in the juice, the possibility of it being affected by the kinds of yeast taking an active part in the fermentation must be considered.

It is well known that different yeasts possess widely different powers of attenuation. Extreme cases could be instanced among the cider yeasts which have been examined at the Institute, and therefore the comparative influence of diverse forms upon the course of fermentation under practical conditions required to be determined.

The behaviour of a large number of different yeasts in sterilised apple juice has been examined. It is not necessary here to refer in detail to the results, since beyond establishing the fact that there is a wide variation in the fermentative powers of many varieties, modified in extent according to the richness or scarcity of nitrogenous yeast food in the juice used, they have little direct bearing upon the problem under consideration.

It is the general custom in cider-making to allow the juice to ferment spontaneously, the usual result being that several different varieties of yeast simultaneously take an active part in the fermentation. As will be shown in a later paper the yeast flora of different ciders varies considerably; and it is therefore essential that there should be some knowledge as to the proper allowance to be made for such variations before a satisfactory conclusion regarding the factors which determine the rate of fermentation can be arrived at.

The examination of the yeast flora of ciders in a state of fermentation shows that there are as a rule one or more species present, which are capable of fermenting the cider to dryness, if the supply of nitrogenous yeast food and other conditions are suitable. The isolation and detailed examination of the different forms is, however, hardly necessary for this purpose, since, if ciders of comparatively high specific gravity in which the fermentation is very slow or has practically ceased are being dealt with, the addition of a small quantity of ammonium tartrate or some other nitrogenous substance which the yeast can assimilate at once leads to an increase in the rate of fermentation; and there is no cessation of fermentation, until the specific gravity is reduced to a low figure, thus proving that at least one of the ferments in the cider is capable of reducing it to dryness under suitable conditions. Examples of this effect have already been given above in Tables C and D in connexion with the consideration of the nitrogenous constituents of the juice.

The general results as a whole appear to indicate that the factor of the amount of nitrogenous yeast-food overshadows entirely the yeast factor, and that there are normally present sufficiently powerful ferments to produce the maximum rate which the nitrogenous contents and other conditions allow. Experiments with "dominant" fermentations by selected yeasts support this view. Several series of these have been carried on during the past four seasons. The results given in the two following Tables, G and H, are typical of all, as far as they illustrate the point in question. In each series fresh juice direct from the press was

used. The bulk required to fill the number of casks used was pumped into a large vessel, and there thoroughly mixed, after which each cask was filled with juice from this vessel and an active culture of one of the selected yeasts added. The quantity of yeast added to each cask was approximately the same, and was sufficient to dominate the character of the fermentation, this being essential since the juice was not sterilised, and therefore contained in a living state the organisms which would in the ordinary course of events set up spontaneous fermentation. In every series one cask was left uninfected, so that the "natural" and "dominant" fermentations could be compared.

TABLE G.

Juice obtained from a mixture of White Jersey and Cap of Liberty apples: pressed Nov. 21st, 1905:

Cask	Selected yeast	Specific gravity						
		Nov. 23	Nov. 30	Dec. 6	Dec. 13	Dec. 20	Jan. 3	Jan. 10
1	Yeast C	1·052	1·047	1·041	1·031	1·024	1·018	1·011
2	,, D	1·052	1·050	1·044	1·033	1·026	1·016	1·011
3	,, E	1·052	1·050	1·045	1·034	1·027	1·017	1·012
4	,, F	1·054	1·051	1·044	1·033	1·026	1·020	1·013
5	,, G	1·052	1·050	1·045	1·036	1·028	1·020	1·012
6	Natural fermentation	1·053	1·050	1·046	1·037	1·031	1·022	1·016

TABLE H.

Juice obtained from a mixture of Cap of Liberty, Yarlington Mill, Royal Wilding, and Strawberry Norman apples: pressed Nov. 26th, 1906:

Cask	Selected yeast	Specific gravity						
		Nov. 26	Dec. 1	Dec. 3	Dec. 4	Dec. 5	Dec. 7	Dec. 28
1	No. 19	1·052	1·052	1·051	1·051	1·050	1·048	1·038
2	,, 39	1·052	1·052	1·052	1·051	1·050	1·046	1·033
3	,, 61	1·052	1·052	1·051	1·050	1·049	1·048	1·036
4	,, 81	1·052	1·052	1·051	1·050	1·050	1·047	1·035
5	,, 119	1·052	1·051	1·051	1·050	1·048	1·047	1·033
6	,, 126	1·052	1·052	1·051	1·051	1·050	1·047	1·035
7	,, 134	1·052	1·052	1·051	1·050	1·049	1·048	1·036
8	,, 138	1·052	1·052	1·051	1·049	1·049	1·048	1·036
9	,, 152	1·052	1·052	1·051	1·049	1·049	1·046	1·036
10	Natural fermentation	1·052	1·052	1·052	1·051	1·050	1·049	1·036

It will be seen from these tables that there was on the whole very little variation in the general course of fermentation of the individual ciders of the respective series: and what differences did occur may have been largely, if not entirely, due to causes other than the yeast influence. Such minor variations regularly occur in adjoining casks of the same cider fermented spontaneously: for which, as will be seen later, the casks themselves are probably largely responsible. It is also to be expected that uninfected ciders would ferment a little more slowly than infected juices, since the addition of an appreciable quantity of active yeast at the outset in the latter cases would naturally not be without some influence upon the course of fermentation, in its earlier stages at any rate. Even although the two ciders used did not differ so widely in their natural rate of fermentation as many fermented spontaneously, there was nevertheless a marked difference as a whole between the respective members of the two series as compared with the slight variations between individual members of the same series. The yeasts selected varied considerably in their powers of attenuation, No. 61 and F especially having comparatively low fermentative power, while No. 39 and D are vigorous forms: but with neither of these extreme forms was the course of fermentation materially altered from the normal spontaneous type. Hence it is clear that at the most the variety of yeast used plays but a secondary part in determining the rate of fermentation; and, bearing in mind the results obtained with the yeasts of limited fermentative power, it seems probable that normally the rate is not far short of the maximum, which the composition of the juice as regards assimilable nitrogenous matter allows.

The influence of aeration.

As shown by Hansen (3) when a free supply of air is given to fermenting liquids an increased multiplication of the yeasts results: while if air is excluded, the rate of multiplication is lowered. The rate of fermentation is thereby affected, being quickened after the aeration of the fermenting liquid.

Cider and perry behave in the same way as other fermentable liquids in this respect, as was shown by the following experiment. A number of narrow-necked bottles were filled to the neck with freshly pressed juice from Spice pears, and placed in an incubator at 27° C. Some were left uncorked and the remainder were corked, a vent tube being passed through the cork and opening under water to allow of the escape of the carbonic acid formed during fermentation. In the former a certain amount of air could come into contact with the juice at the

exposed surface in the neck, although since the necks were narrow, the exposed surface was, relatively to the bulk of the liquid, exceedingly small. In the latter no air could come into contact with the juice. The daily records of the specific gravities were as under:

TABLE I.

	Uncorked bottles	Corked bottles
After 1 day	1·047	1·047
,, 2 days	1·046	1·045
,, 3 ,,	1·040	1·043
,, 4 ,,	1·033	1·040
,, 5 ,,	1·026	1·036
,, 6 ,,	1·020	1·033
,, 7 ,,	1·016	1·030
,, 8 ,,	1·013	1·028
,, 9 ,,	1·011	1·027

The influence of the access of air was thus most marked in spite of the very limited exposed surface.

Similar experiments were carried out in 30 gall. casks in the cider house on the juice of mixed apples. Three casks, A, B and C, were filled to the bunghole with juice, which had previously been thoroughly mixed in a larger vessel. Over the bunghole of cask A a sheet of brown paper was pasted, and a pinhole made in it to allow of the escape of the gas given off by the fermenting liquor. No air could thus gain access to the cider. The bunghole in cask B was loosely plugged with a bung of sacking, so that not only could the carbonic acid gas escape easily, but also a limited quantity of air could find its way to the surface of the cider. Cask C was left unbunged, so that air could reach the surface of the cider exposed at the bunghole. The weekly records of the specific gravities were as under:

TABLE J.

	Cask A	Cask B	Cask C
When experiment started	1·046	1·046	1·046
After 1 week	1·046	1·044	1·041
,, 2 weeks	1·043	1·040	1·039
,, 3 ,,	1·042	1·038	1·036
,, 4 ,,	1·037	1·030	1·025
,, 5 ,,	1·031	1·024	1·019
,, 6 ,,	1·027	1·017	1·012
,, 7 ,,	1·022	1·013	1·008
,, 8 ,,	1·017	1·009	1·007
,, 9 ,,	1·011	1·007	1·006

In this series, again, although the final point reached was not very different, there was a considerable variation in the rates of fermentation, C fermenting most quickly, B next, and A slowest, the order thus corresponding with the amounts of air which could reach the respective juices.

A similar influence of air is noticeable in another connexion. It is the practice of certain makers to "keeve" the juice before placing it in the fermenting cask. In other words, the juice is pumped direct from the press to large open wooden vessels, the "keeves," in which it is allowed to remain as long as it will throw up solid matter in the "head" which is formed on its surface, the object of the process being to clear the juice quickly of its suspended solid matter. Other makers prefer to place the juice at once into the fermenting casks, which are ordinary casks standing on their sides, so that the froth and other matter thrown up in the "head" can work out at the bunghole. Several comparative trials of the two methods have been made at the Institute and it has been noticed on every occasion that, using the same kind of juice, fermentation starts sooner and proceeds for a time more rapidly in the "keeved" than in the "unkeeved" juice. For example, the juice from Oldfield pears, when keeved, dropped in specific gravity from 1·070 to 1·062, while the same juice in cask, in the same period of time dropped only from 1·070 to 1·069. Similarly the keeved juice of Cummy Norman apples showed a drop in specific gravity from 1·058 to 1·039 as compared with a fall from 1·058 to 1·049 in the case of the unkeeved juice. Seeing that there is a large surface of juice exposed to the air in the "keeves," while there is only the very limited area of the bunghole exposed with the "unkeeved" juice, and that the other conditions are similar, it seems justifiable to conclude that the more rapid fermentation of the "keeved" juice is due to the greater exposure to air.

The influence of temperature.

That the temperature at which fermentations are conducted has an important influence upon the rate is well known. For every yeast capable of inducing fermentation there are maximum and minimum temperature limits above and below which respectively no fermentation occurs: and there is also an optimum temperature at which fermentation proceeds most rapidly. In the case of cider fermentations there seems to be no departure from the general custom in this respect; and

there is no occasion therefore to go into detail on the subject. The following figures suffice to give a fair idea of the effect of temperature, and to illustrate that the comparative rates of fermentation of different ciders are much the same at a high as at a low temperature.

TABLE K.

	Cider, Counsellors			
Temp.	at ordinary temperature		at 25° C.	
Sp. gr.	after 1 week	1·067	after 1 day	1·068
,,	,, 2 weeks	1·063	,, 2 days	1·062
,,	,, 3 ,,	1·038	,, 3 ,,	1·041
,,	,, 4 ,,	1·014	,, 4 ,,	1·020
,,	,, 5 ,,	1·008	,, 5 ,,	1·009
	Cider, Cap of Liberty			
,,	after 1 week	1·061	after 1 day	1·062
,,	,, 2 weeks	1·059	,, 2 days	1·055
,,	,, 3 ,,	1·049	,, 3 ,,	1·049
,,	,, 4 ,,	1·041	,, 4 ,,	1·043
,,	,, 5 ,,	1·037	,, 5 ,,	1·038
	Cider, Horners			
,,	after 1 week	1·053	after 1 day	1·051
,,	,, 2 weeks	1·049	,, 2 days	1·048
,,	,, 3 ,,	1·046	,, 3 ,,	1·047
,,	,, 4 ,,	1·042	,, 4 ,,	1·046
,,	,, 5 ,,	1·040	,, 5 ,,	1·041

Summary and Conclusions.

The results of the work may be briefly summarised as follows:

(*a*) The rate of fermentation of ciders and perries made from different varieties of vintage fruit varies considerably.

(*b*) There is probably a relation between the rate of fermentation and the variety of fruit from which the cider or perry is made. Certain varieties, for instance, as a rule yield juices which ferment slowly, while others give juices which generally ferment at a rapid rate.

(*c*) The main factor in determining the rate of fermentation appears to be the nitrogenous matter present in the juice, which is assimilable by the yeast. The quantity of such substances present is generally insufficient for satisfactory nutrition of the yeast, and consequently the rate of fermentation is normally slower than would be the

case if sufficient nitrogenous food were present to meet the full requirements of the yeast. The relative rates of fermentation of ciders and perries fermented under similar conditions are probably an index of the relative amounts of assimilable nitrogenous matter present.

(*d*) The state of ripeness of the fruit at the time of milling affects the rate of fermentation of the juice. During the course of ripening the rate becomes slower until a certain point is reached, which probably represents the period of perfect maturity of the fruit. Afterwards the rate increases in proportion as ripeness proceeds to decay. The degree of exposure of the fruit to direct sunlight also affects the rate of fermentation, the more exposed the fruit the slower the rate. In each case the result appears to be due to the quantity of assimilable nitrogenous matter in the juice being influenced.

(*e*) Direct influence on the rate of fermentation of the juice by any of the chemical constituents other than the nitrogenous substances has not been observed. If any have an influence, it is masked by other factors of greater importance. An indirect influence on the rate by the mucilaginous elements is occasionally met with, due to the formation of a clot which mechanically impedes the action of the organisms of fermentation.

(*f*) The rate of fermentation in practical cider-making does not appear to be materially affected by the fermentative powers of the kinds of yeast present in the juice. Normally there are present varieties which are capable of maintaining the fermentation at practically the maximum rate allowed by the nitrogenous constitution of the juice. "Dominant" fermentation with selected yeasts of high or low fermentative powers had comparatively little effect upon the rate.

(*g*) The aeration of the juice has a marked effect upon the rate of fermentation, the admission of air to the juice producing a decided increase in the rate.

(*h*) The temperature at which the fermentations are conducted affects the rate in the customary manner.

It is clear, therefore, that apart from the use of purely practical methods, *e.g.* filtration,—which it is not intended to consider here—a certain measure of control over the rate of fermentation of ciders and perries can be exercised by the cider maker, and that therefore the production of sweet and dry types of these beverages need not be more or less haphazard, as is commonly the case. By careful selection of the varieties of fruit used and by suitable blending of various types, combined with attention to the condition of ripeness of the fruit at the time of making, it should be possible to obtain a juice

possessing the desired rate of fermentation, although some allowance for seasonal influences is necessary. These have not been considered above, as the work has not been extended over a sufficiently long period to allow of definite conclusions being drawn. At the same time it is fairly established that in some seasons the average rate of fermentation is much faster than in others. It would appear from the results as to the effect of direct sunlight as though the amount of sunshine during the period of ripening of the fruit upon the trees played an important part in seasonal influence. During the course of fermentation of the liquors the rate may be controlled to some extent by aeration and temperature.

Although the subject has been considered almost entirely from the point of view of the rate of fermentation, it should be mentioned that not only the rate but also the degree to which fermentation proceeds is involved. Although perhaps not invariably the case, as a rule fermentation can proceed to a further point in rapidly than in slowly fermenting juices. Accordingly no distinction has been made between them. In some cases the latter feature would more correctly express the facts than the former.

In conclusion I take this opportunity of expressing my indebtedness to Mr James Watts for his kindness in placing his factory at my disposal for experiments with selected yeasts; to the many cider makers and others, who have rendered considerable assistance in the direction of obtaining different varieties of vintage fruit required for the work; and to the various members of the staff of the Institute, who have carried out the practical work in the cider house.

THE NATIONAL FRUIT AND CIDER INSTITUTE,
LONG ASHTON, BRISTOL.

BIBLIOGRAPHY.

1. W. B. ALLWOOD. The Chemical Composition of Apples and Cider. *U.S. Depart. of Agriculture, Bureau of Chemistry, Bulletin* No. 88.
2. B. T. P. BARKER. *Reports on Cider-making*, 1904—5, 5—6, 6—7, and 7—8. *Annual Reports of the National Fruit and Cider Institute*, 1905, 1906, 1907 and 1908. (Also published in the *Bath and West and Southern Counties of England Society's Journal*, 1905—6—7 and 8.)
3. E. C. HANSEN. *Compt. rend. de Carlsberg*, 1879, Bd. 1.
4. HOGG AND GRAVES BULL. *The Herefordshire Pomona.*
5. KULISCH. *Beiträge zur Kenntniss der chem. Zusammensetzung der Äpfel. Landw. Jahrb.* 1892.
6. F. J. LLOYD. *Report on Cider-Making. Board of Agriculture and Fisheries*, 1903.
7. TRUELLE. *Les meilleurs fruits de pressoir*, 1895.

THE MINERAL CONSTITUENTS OF FOODS.

By HERBERT INGLE, B.Sc., F.I.C., F.R.S.S.A., F.C.S.,

Late Chief Chemist, Transvaal Department of Agriculture.

THE importance of a "well balanced" ration in feeding animals has long been recognised, and most intelligent stock-keepers pay some attention to the albuminoid ratio of the foods they use.

That the requirements of animals with respect to the proportions of albuminoids or "flesh-formers" to fats and carbohydrates or "heat-formers" in their diet vary with circumstances is also realised by many, and proper attention is paid to this in framing rations for various animals kept under different conditions. But beyond the general and vague belief that the foods should contain a sufficient amount of "ash constituents" or "bone-formers," little consideration has been given to the diet of animals with respect to its mineral constituents.

The author has recently[1] been investigating from a chemical aspect, a disease of the bones of horses, donkeys, and mules, which is particularly prevalent in South Africa, and from a consideration of the results obtained has been led to a theory which, he ventures to think, may account for the prevalence of the disease in that country, and may indicate means for its prevention, or at least amelioration, and at the same time furnish points worthy of consideration by all interested in the feeding of animals.

The disease in question, Osteoporosis, is characterised by an extraordinary weakening of the bones, and is the cause of considerable losses among stable-fed horses and mules.

In 1905, the writer examined the bones of a considerable number of horses, mules and donkeys. The bones were merely numbered and

[1] v. "Osteoporosis in Animals," *Journal of Comparative Pathology and Therapeutics*, March, 1907.

he was not informed which were from diseased and which from healthy animals. When the analyses were finished he found it possible to accurately divide the bones, from their chemical composition, into those from healthy and those from diseased animals.

Those from diseased animals were much richer in organic matter and poorer in ash than those from healthy animals. The most conspicuous difference was shown by taking the ratio of nitrogen (which is a measure of the nitrogenous substance—ossein—present) to ash in the bones, as the influence of the very variable fat present was thus eliminated.

The value of this ratio was found to vary in the case of diseased animals from 1 : 9·8 to 1 : 11·7, the mean value being 1 : 10·8; with the healthy animals the ratio varied from 1 : 13·5 to 1 : 15·6, the mean being 1 : 14·37.

In the bones of healthy animals the mean amounts of lime and phosphorus pentoxide were 32·28% and 21·41% respectively, while with the diseased bones the figures were 28·50 and 19·06%.

In tabulating the results and considering the possible causes which might tend to produce such a condition of the bones, the author was led to the conclusion that the peculiar diet of working animals in the country—a ration composed wholly of cereals, either oat-hay or oat-hay and "mealies" (*i.e.* maize or Indian corn)—might possibly account for the frequency of the disease in South Africa.

Some veterinary surgeons ascribe the prevalence of the disease to deficiencies of the food-stuffs in lime and phosphoric acid, while others are persuaded that the disease is caused by a specific micro-organism and is of an epidemic character, though no organism has been found, nor can the disease be transmitted by inoculation or administration of diseased bone.

Now the proper ratio of phosphorus pentoxide to lime in the food of animals in order to favour bone formation and renewal, has not been directly determined, but may be deduced from one or two considerations. Thus, the milk of animals may be assumed to contain these ingredients in proper proportion for the needs of young animals.

Cows' milk contains on the average, about 0·17 per cent. of phosphorus pentoxide and 0·15 per cent. of lime, *i.e.* in the ratio of 100 of phosphorus pentoxide to 89 of lime. In the bones of animals the two substances are present in the ratio of about 100 of phosphorus pentoxide to 150 of lime.

According to Rothamsted experiments, the amounts of the two

substances present in 1000 lbs. of the whole bodies of animals are as follows:

	Phosphorus pentoxide	Lime	Ratio
Fat calf	15·35	16·46	100 : 108
Half fat ox	18·39	21·11	100 : 116
Fat lamb	11·26	12·81	100 : 114
Store sheep	11·88	13·21	100 : 112
Store pig	10·06	10·79	100 : 107
Fat pig	6·54	6·36	100 : 95

Remembering that some of the phosphoric acid of the ash is present in the food in the form of organic matter and is utilised in the animal in the formation of tissue other than bone (*e.g.* brain) and to a greater extent than lime, it may probably be assumed that the food of an animal should contain about equal parts of phosphorus pentoxide and lime in its ash.

In the two substances which form the staple diet of horses and mules in S. Africa—oat-hay and mealies—lime and phosphorus pentoxide are present in the ash in the following proportions:

	Phosphorus pentoxide	Lime
Oat-hay	100	77
Mealies (maize grain)	100	4

These are calculated from the ash analyses of Wolff. In South African grown oat-hay, I find that both the lime and phosphorus pentoxide present are smaller than in the average of European samples and that on the average the ratio is 100 : 51.

From the figures given by Warington for the lime and phosphorus pentoxide in the whole of the oat-crop, the ratio is 100 : 60.

It is evident from the above figures that the usual South African diet for working animals does not supply lime and phosphorus pentoxide in the proportions which we have adduced reasons for believing are best adapted for the nutrition of bone. On the contrary, a ration consisting of oat-hay and mealies provides a large excess of phosphorus pentoxide over lime.

As to the injurious effect of the prolonged use of such a diet upon horses and mules, we have no records of direct experiments having been made, but in 1891, Weiske[1] experimented with rabbits on these lines.

Adult rabbits, from the same litter, were divided into four lots and fed for three months upon:

1. Hay.
2. Mixture of hay and oats.

[1] *Landwirtschaftliche Versuchs-Stationen*, xxxix. 241—268.

3. Oats alone.

4. Oats to which sodium dihydrogen phosphate was added (so as to increase artificially the ratio of phosphoric acid to lime in the food).

It was noticed that the urine of rabbits of lots 1 and 2 was alkaline while that of lots 3 and 4 was strongly acid. At the end of the period the rabbits were killed, weighed and their skeletons cleaned and weighed.

The results were as follows, the weights being in grammes:

	Lot 1	Lot 2	Lot 3	Lot 4
Weight of bodies	2430	3420	2030	1810
Weight of skeletons ...	87·66	115·80	69·28	63·76

The bones of lots 1 and 2 were heavier, stronger and richer in ash than those of lots 3 and 4. Those of lot 4 were very thin and breakable and contained a smaller proportion of lime and phosphoric acid than the others.

In a later paper (1894), Weiske records experiments in which the effects upon the bones of animals feeding upon oats alone were successfully neutralised by the addition of carbonate of lime to the diet.

Another point of importance may be here pointed out—the erroneous idea that bran, which is almost universally regarded as being particularly rich in "bone-forming material," *i.e.* ash, is useful in aiding bone formation. From the point of view now under consideration, bran would be a particularly bad food, as the ratio of lime to phosphorus pentoxide is extremely low—the actual proportions present being approximately 0·3 per cent. of lime to 3·3 per cent. of phosphorus pentoxide, or in the ratio of 9 : 100. This is confirmed by the occurrence of a bone disease, known as "bran rachitis," "bran disease" or "miller's horse rickets" which is observed in animals fed largely on a bran diet.

It is thus evident that, whether osteoporosis be due to a specific organism or not, a condition of the bones of animals similar to that which results from the disease, may be induced by the use of a diet containing a low ratio of lime to phosphorus pentoxide.

It may be well to give a table showing the ratio of lime to phosphorus pentoxide in the ash of some typical foods. Ignoring for the moment[1] the actual quantities of these constituents and giving only the ratios in which they occur, the following table has been prepared from analyses of average samples as given by Wolff and Warington:

[1] See Appendix for actual composition of many food-stuffs.

Food-stuff	Ratio Phosphorus Pentoxide		Lime
Lucerne hay	100	:	478 (Wolff)
Crimson clover hay	100	:	445 ,,
Red clover hay	100	:	361 (Warington)
,, ,,	100	:	359 (Wolff)
Meadow hay	100	:	262 (Warington)
,,	100	:	247 (Wolff)
White clover hay	100	:	227 ,,
Oat straw	100	:	181 ,,
Oat grain	100	:	16 ,,
Oats (whole plant, green)	100	:	77 ,,
Oats (,, ,, ripe)	100	:	62 (Warington)
Barley (whole plant)	100	:	44 ,,
Mealies or maize (grain)	100	:	4 (Wolff)
Wheat bran	100	:	9 ,,
Linseed cake	100	:	24 ,,

While the following are the ratios calculated from our analyses of South African grown produce:

	Phosphorus pentoxide		Lime
Oat hay (Malmesbury, Cape Colony)	100	:	23
,, (Middelburg, ,,)	100	:	57
,, (Harmon, ,,)	100	:	65
,, (Magaliesberg, Transvaal)	100	:	44
,, (Pretoria, ,,)	100	:	62
,, (Potchefstroom, ,,)	100	:	53
Mean	100	:	51
Rhodes grass hay (*Chloris guyana*)	100	:	250
Sweet grass hay (*Chloris virgata*)	100	:	139
Boer manna hay (*Setaria italica*)	100	:	94
Blue grass hay (*Andropogon hirtus*)	100	:	168
Teff grass hay (*Eragrostis Abyssinica*)	100	:	125
Veld hay (mixed grasses)	100	:	320
Teosinte hay (*Euchloena Mexicana*)	100	:	203
Golden millet hay (*Setaria sp.*)	100	:	88
Californian green moha (*Setaria sp.*)	100	:	137
Broom corn millet (*Panicum crus-galli*)	100	:	174
Lucerne hay (*Medicago sativa*)	100	:	431
Cow-pea hay (*Vigna catjang*)	100	:	248
Velvet bean hay (*Mucuna utilis*)	100	:	581
Maple pea hay (*Pisum arvense*)	100	:	202
Mealie stalks (*Zea mays*)	100	:	136
Kaffir corn stalks (*Sorghum*)	100	:	100
Millet stalk	100	:	67
Oat straw	100	:	209
Wheat straw	100	:	250
Tall fescue grass (*Festuca elatior*)	100	:	258
Burnet, green (*Poterium sanguisorba*)	100	:	485
Sheep's parsley, green (*Petroselinum sativum*)	100	:	312
Prickly pear "leaves" (*Opuntia ficus indica*)	100	:	1260

There can be little doubt that animals may be gradually accustomed to live upon a diet that is at first unsuited to their requirements, and I have every reason to believe that the South African bred horse is less liable to succumb to osteoporosis, or to suffer from deficiencies in the composition of the ash of his food, than are imported animals.

Interesting accounts of outbreaks of osteoporosis among imported horses, donkeys and mules in 1898 and in 1904 at the Military camps of Wynberg and Middelburg in Cape Colony are given by Capt. Lane[1], who particularly noticed the improvement effected in the diseased animals by a change in diet from oat-hay, mealies and bran to one containing lucerne, green forage and bone meal. These cases afford strong confirmation of the success of the treatment which the theory here adduced would indicate as beneficial, though the treatment was adopted rather with the object of increasing the amount of both phosphoric acid and lime in the food, than of increasing the ratio of the latter to the former (which indeed was, in my opinion, the cause of its success).

It will be seen from the above considerations that the writer is of opinion that it is not the poverty of South African grown produce in lime and phosphoric acid (as compared with European grown food-stuffs of the same kind) which is to be blamed for the prevalence of bone troubles among animals there, but rather the practice of feeding such animals exclusively upon a cereal diet.

Probably in Europe, if horses and mules were fed entirely upon oat-hay, similar diseases would result, though there is some evidence that in certain districts in Africa, both the soil and the crops grown on it are poorer in lime than the corresponding crops grown elsewhere. Our analyses of Transvaal soils indicate that they are, as compared with English soils, very poor in phosphoric acid, nitrogen and lime, but usually rich in potash.

Now to plants, phosphoric acid is apparently more important than lime, at least so far as seed formation is concerned, and the yield of seed is often limited by the amount of phosphoric acid available.

In many parts of South Africa, it has been the practice to attempt to compensate for the assumed deficiency in lime and phosphates of the usual food-stuffs given to animals, by the administration of bone meal, and "sterilised bone meal" is largely used for the purpose. Such a practice undoubtedly tends to mitigate the evil alluded to, for we may take it that bone meal contains lime and phosphoric acid in approximately the correct proportion for bone nutrition.

But to add a material containing the two substances in correct ratio, to a food which otherwise is far too rich in phosphoric acid, though it improves the final ratio in the mixture, is not so satisfactory a method as the substitution for a portion of the oat-hay of a food-stuff relatively

[1] *Veterinary Journal*, May 1906, p. 232.

rich in lime—*e.g.* a leguminous fodder-crop like lucerne or cow-peas. Moreover, the more extended use of leguminous foods would improve the rations of animals in other ways, notably by narrowing the albuminoid ratio.

In conclusion, I would urge the importance of giving due consideration, in framing rations for animals, to the amount and *composition* of the ash of the foods, for the supply of materials for the proper development of bone, and of the mineral constituents necessary for vital processes, are of as much importance to the well-being of the animals as that of proteids, carbohydrates and fats in appropriate quantities. Where a considerable variety of food-stuffs is employed, *e.g.* in England, the probability of much injury being done by ignoring this aspect of the question is not nearly so great as when two or three constituents only enter into the ration, but even in such cases, a proper recognition of the points I have raised in this paper would probably often be useful.

The writer is fully convinced that if due regard were paid to these points and a more varied diet were supplied to horses and mules in South Africa, there would be a marked improvement in the health and well-being of the draught animals, and that in time horses of greater weight of bone would probably be reared.

Possibly the same arguments may apply to cattle[1], but as a rule the ox is allowed to graze and thus obtain greater variety of diet, so that its needs in this connexion are probably not so great as with stall-fed animals.

I would take this opportunity of acknowledging the help of my staff in the carrying out of the analyses which are given in the appendix.

[1] An investigation, from the chemical standpoint, of a peculiar bone disease, prevalent in certain districts of the Transvaal, among cattle and known as "Stiff-sickness," has been commenced.

APPENDIX.

COMPOSITION OF CERTAIN TRANSVAAL GROWN FOODS.

Green Forage.

	Tall fescue (*Festuca elatior*).	Burnet (*Poterium sanguisorba*).	Sheep's parsley (*Petroselinum sativum*).	Prickly pear (*Opuntia ficus indica*).
Moisture	60·69	61·56	75·83	93·79
Ash	4·08	3·59	3·19	1·13
Protein	5·90	5·64	5·43	0·42
Soluble carbohydrates	16·75	21·56	11·95	3·89
Ether extract	4·64	2·09	0·72	0·12
Crude fibre	7·94	5·56	2·88	0·65
	100·00	100·00	100·00	100·00
Albuminoid ratio	1 : 4·8	1 : 4·7	1 : 2·5	1 : 10·0
The ash included:				
Silica	1·51	0·80	0·32	0·003
Potash	1·55	0·77	1·01	0·34
Lime	0·31	0·63	0·50	0·29
Phosphorus pentoxide	0·12	0·13	0·16	0·023
Ratio:				
100 of P_2O_5 to lime	258	485	312	1261

Hays.

	Oat hay (*Avena sativa*). Potchefstroom	Blue grass hay (*Andropogon hirtus*). Natal	Teff hay (*Eragrostis Abyssinica*). Standerton
Moisture	8·00	7·98	8·88
Ash	4·23	5·96	5·55
Protein	5·65	4·38	6·21
Soluble carbohydrates	44·03	41·87	37·49
Ether extract	3·87	1·31	2·80
Crude fibre	34·22	38·50	39·07
	100·00	100·00	100·00
Albuminoid ratio	1 : 9·4	1 : 10·2	1 : 7·1
The ash contained:			
Silica	2·01	3·50	3·25
Potash	—	1·04	1·28
Lime	0·18	0·47	0·30
Phosphorus pentoxide	0·34	0·28	0·24
Ratio:			
100 of P_2O_5 to lime	53	168	125

Hays (continued).

	Rhodes grass hay (*Chloris guyana*). Pretoria	Boer manna (*Setaria italica*). Witwatersrand	Californian green moha. Pretoria
Moisture	8·99	8·25	7·97
Ash	8·72	7·78	9·27
Protein	9·19	5·00	10·52
Soluble carbohydrates	29·27	46·24	35·62
Ether extract	1·35	1·88	1·22
Crude fibre	42·48	30·85	35·60
	100·00	100·00	100·00
Albuminoid ratio	1 : 3·5	1 : 10·1	1 : 3·7
The ash contained:			
Silica	4·00	5·67	3·71
Potash	1·15	—	2·44
Lime	0·60	0·30	0·41
Phosphorus pentoxide	0·24	0·32	0·30
Ratio:			
100 of P_2O_5 to lime	250	94	137

	Japanese broom corn hay (*Panicum crus-galli*). Pretoria.	Golden millet hay (*Setaria sp.*). Pretoria.	Cow-pea hay (*Vigna catjang*). Pretoria.
Moisture	9·65	7·88	8·21
Ash	8·76	9·49	6·08
Protein	6·83	11·11	13·21
Soluble carbohydrates	38·84	29·54	39·59
Ether extract	1·16	0·95	2·40
Crude fibre	34·76	41·03	30·51
	100·00	100·00	100·00
Albuminoid ratio	1 : 6·1	1 : 2·9	1 : 3·4
The ash contained:			
Silica	4·80	1·54	0·69
Potash	1·92	5·16	1·56
Lime	0·33	0·35	1·56
Phosphorus pentoxide	0·19	0·40	0·63
Ratio:			
100 of P_2O_5 to lime	173	88	248

	Teosinte hay (*Euchloena Mexicana*). Pretoria.	Velvet bean hay (*Mucuna utilis*). Potgietersrust.	Vetches (*Vicia villosa*).
Moisture	11·45	9·25	9·60
Ash	9·73	7·83	8·62
Protein	7·89	13·30	20·56
Soluble carbohydrates	33·02	39·44	35·98
Ether extract	1·52	2·55	4·01
Crude fibre	31·39	27·63	21·23
	100·00	100·00	100·00
Albuminoid ratio	1 : 5·3	1 : 3·4	1 : 2·2
The ash contained:			
Silica	3·25	1·60	0·89
Potash	4·10	2·21	4·74
Lime	0·59	1·80	0·88
Phosphorus pentoxide	0·29	0·31	0·67
Ratio:			
100 of P_2O_5 to lime	203	580	131

	Blue lupines (*Lupinus angustifolius*).	White lupines (*L. albus*).	Lucerne (*Medicago sativa*).
Moisture	8·18	7·79	7·97
Ash	8·32	7·84	8·94
Protein	17·06	14·09	15·49
Soluble carbohydrates	41·72	50·10	30·58
Ether extract	2·68	2·75	2·26
Crude fibre	22·04	17·43	34·76
	100·00	100·00	100·00
Albuminoid ratio	1 : 2·8	1 : 4·0	1 : 2·3
The ash contained:			
Silica	0·40	0·90	0·49
Potash	1·41	2·82	3·61
Lime	3·83	0·91	1·38
Phosphorus pentoxide	0·65	0·43	0·32
Ratio:			
100 of P_2O_5 to lime	589	212	431

	Maple pea (*Pisum arvense*).	Veld hay (mixed grasses).	Sweet grass hay (*Chloris virgata*).
Moisture	8·04	8·07	7·52
Ash	6·92	5·44	8·13
Protein	16·27	3·43	7·61
Soluble Carbohydrates	35·25	43·84	37·57
Ether extract	2·36	1·21	1·04
Crude fibre	31·16	38·01	38·13
	100·00	100·00	100·00
Albuminoid ratio	1 : 2·5	1 : 13·6	1 : 5·3
The ash contained:			
Silica	0·77	4·07	3·44
Potash	3·49	0·59	2·57
Lime	0·99	0·32	0·25
Phosphorus pentoxide	0·49	0·10	0·18
Ratio:			
100 of P_2O_5 to lime	202	320	139

Miscellaneous.

	Brewers' grains.	Bran.
Moisture	77·64	11·02
Ash	1·01	6·06
Protein	7·26	19·25
Carbohydrates	9·66	54·23
Ether extract	1·02	2·46
Crude fibre	3·41	6·98
	100·00	100·00
The ash contained:		
Silica	0·42	0·07
Lime	0·07	0·16
Phosphorus pentoxide	0·34	2·90
Ratio:		
100 of P_2O_5 to lime	21	5·5

THE ACTION OF HEAT AND ANTISEPTICS ON SOILS.

By SPENCER UMFREVILLE PICKERING, M.A., F.R.S.

In a previous communication (Vol. II. p. 411) it was shown that those organic constituents of a soil which are rendered soluble when it is heated, produce an inhibitory effect on the germination of seeds, the percentage of seeds germinating being reduced, and the time of their incubation being increased, this increase being, moreover, directly proportional to the amount of organic matter and of nitrogen rendered soluble by the heating. The formation of the inhibitory substance begins at temperatures as low as 30°, and attains a maximum at about 250°. Bacteria, apparently, exercise no influence on germination.

It was suggested that the increased growth and nitrogen-absorption exhibited by non-leguminous plants in heated soils might equally be explained by the chemical alteration produced by heat, without attributing it to the accompanying bacterial changes. So far as can be judged from the inadequate data available, the actual increase in nitrogen rendered soluble by heating to 90°—100° is sufficient to account for the extra nitrogen assimilated by the plants in such experiments as those of Darbishire and Russell (II. 316), and in those of the writer with apple trees (II. 412)[1]; though in view of the changes progressing in the soil during the period of growth, and from other considerations, no great stress can be laid on the concordance or non-concordance of these two quantities. Certainly, the general character of the results is indicative of their being due to the presence of

[1] Thus, on heating Harpenden soil at 100° there was an increase of ·004 per cent. in soluble nitrogen (II. 422), which would represent 0·64 grams in the 16 kilos. of soil used in each of Darbishire and Russell's experiments; whereas, in the only case where data are given, the increased absorption by the crop was 0·65 grams (II. 316, Table IX). In the case of the apple trees, the extra absorption, as compared with extra nitrogen available, was considerably less.

an extra supply of available food material in the soil to start with, rather than to any permanent alteration affecting the rate at which the nitrogen is rendered available; for the phenomenon of increased growth disappears under continuous cropping, but may be made to reappear on repeating the heating (*loc. cit.* p. 318, Table X (*c*)).

That the nitrogenous substance rendered soluble by heating should be favourable to the growth of plants, whilst it is unfavourable to the germination of seeds, does not necessarily present any difficulty, especially as it has been shown that heated soil becomes modified by being kept for some days in a moist condition at summer temperatures, and loses part of its inhibitory properties.

On the other hand, a strong argument that bacterial alteration is an important factor in the behaviour of heated soils, is based on the fact that soils treated with antiseptics behave in a precisely similar, though less energetic manner, their oxygen absorption (which is taken to be a measure of their bacterial condition) being increased, as well as their power of stimulating growth and of increasing the nitrogen absorbed by plants.

The assumption that volatile antiseptics can not alter the chemical condition of the soil (*loc. cit.* p. 323) appears, at first sight, to be acceptable, but, when it is considered that they must owe their antiseptic and anæsthetic properties to chemical changes brought about in living matter, this assumption becomes unjustifiable, and it is evident that the question should be submitted to the test of direct experiment, before any arguments are based upon it. As pointed out by the writer (*loc. cit.* p. 432), the excess of growth of plants in soils treated with antiseptics and in soils heated to 92·5°, is, according to Darbishire and Russell's results, in the proportion of 29 to 215 (though the results available are not strictly comparable), and, if the increase in growth follows the same course as the increase in the time of incubation of seeds, the treated soils would be analogous to soil heated to about 57°, which corresponds with only a small increase in the soluble organic matter.

The existence of such a change has been fully established by the experiments described below, and, at the same time, it has been shown that these treated soils behave towards the germination of seeds in the same way as heated soils do.

Treatment of soils with antiseptics.

In the first series of experiments the sample of Harpenden soil designated by C in the preceding communication, was used (pp. 417, 422, 427). It was from land which had been old pasture up to 1893, and had since been occupied by fruit trees, without any dressings of manure having been given to it since 1896. It had 4 per cent. of pure chalk added to it, and then contained 13·5 per cent. of water. The total nitrogen present was 0·25 per cent. (p. 427), and the soluble nitrogen ·004 per cent. (p. 422).

Six lots of 1 kilo. each were put into similar bottles, and 10 c.c. of carbon disulphide, chloroform, ether and benzene (specially purified) were, respectively, added to four of the lots, the first and last lots remaining untreated. After two hours, each lot was exposed to the air in a thin layer for another two hours, although from 10 to 40 minutes was sufficient to get rid of all smell of the antiseptic. 180 grms. of each was then shaken up for two hours with 1200 c.c. of water, the solutions were filtered, first through paper, and then through a Berkefeld filter; after which 500 c.c. of the filtrate was evaporated in platinum, dried at 100°, weighed, ignited, and weighed again. All the operations with the six lots were carried out simultaneously, except the final filtrations and evaporations, which had to be done successively, one blank being done first, the other last.

Benzene was used in preference to toluene, on account of its volatility, as it was desirable to minimise the time expended in the operations, so that there should be as little opportunity as possible for bacterial change after the soils had been treated. The germination experiments were started within 18 hours of the soils being treated.

The analytical results are given in the first part of Table I. The error in determining the organic matter is at least double that in determining the total extract, as it depends on double the number of weighings, and, since the differences to be considered are very small, it is best, in this instance, to confine our attention to the latter.

As will be seen, every one of the four treated soils yields more extract than either of the untreated ones, the increase varying between 1 and 8 per cent. of the total soluble matter, according to the antiseptic used.

The effect of heat on this same sample of soil was determined by other experiments, entered next in the table—the heating being done, as in the previous work, in closed vessels—and, on plotting out these

TABLE I. *Soluble matter in treated and untreated soils.*

Treatment	Total soluble matter			Soluble organic matter		
	Per cent.	Increase	Temp. equiv.	Per cent.	Increase	Temp. equiv.
Harpenden Soil C, May 16						
Untreated A	·1603	—	—	·0778	—	—
B	·1591	—	—	·0740	—	—
Mean	·1597	—	—	·0759	—	—
Carb. disulph.	·1716	·0119 = 8 p.c.	77°	·0827	—	—
Chloroform	·1648	·0051 = 3 ,,	64° 69°	·0747	—	—
Benzene	·1637	·0040 = 2·5 ,,	62°	·0758	—	—
Ether	·1614	·0017 = 1 ,,	44°	·0764	—	—
Mean	—	3·8 ,,	*66°*	—	—	—
Effect of Heat on the same soil						
Heated at 60°	—	— 2·2 p.c.	—	—	—	—
,, ,, 100°	—	— 24·8 ,,	—	—	—	—
Harpenden Garden Soil, June 16						
Untreated A	·2151	—	—	·0856	—	—
B	·2228	—	—	·0977	·0929	—
C	·2290	—	—	·0953	—	—
Mean	·2223	—	—	·0929	—	—
Carb. disulph.	·2726	·0503 = 23 p.c.	75°	·1372	·0443 = 48 p.c.	74°
Chloroform	·2509	·0376 = 12 ,,	66°	·1134	·0205 = 22 ,,	65°
Benzene	·2402	·0179 = 8 ,,	63°	·0978	·0049 = 5 ,,	47°
Mean	—	*14* ,,	*65°*	—	*25* ,,	*66°*
Ditto, July 20						
Untreated A	·2056	—	—	·0970	—	—
B	·2037	—	—	·0890	·0932	—
C	·2242	—	—	·0937	—	—
Mean	·2112	—	—	·0932	—	—
Carb. disulph.	·2370	·0258 = 14 p.c.	68°	·1093	·0161 = 17 p.c.	62°
Chloroform	·2198	·0086 = 4 ,,	59°	·0942	·0010 = 1 ,,	35°
Benzene	·2157	·0045 = 2 ,,	44°	·1035	·0103 = 11 ,,	58°
Mean	—	*7* ,,	*61°*	—	*10* ,,	*56°*
Effect of Heat on the same soil, July 19						
Unheated (30°)	·2223	—	—	·0929	—	—
Heated at 60°	·2370	·0147 = 6·6 p.c.	—	·1054	·0125 = 13·4 p.c.	—
,, ,, 100°	·4598	·2375 = 107 ,,	—	·2764	·1835 = 198 ,,	—

results, a curve is obtained from which readings may be taken showing to what temperature the soil would have to be heated to give the same increase in soluble matter as that caused by the treatment with antiseptics. These readings are entered as the "temperature equivalents" in the fourth column. They give, as a mean, 66° as the temperature equivalent of these four antiseptics, a value which is fairly close to that (57°) based roughly on Darbishire and Russell's results on the growth of plants. Carbon disulphide gives appreciably higher, and ether lower, results, than the other substances.

TABLE II. *Relative times of incubation of seeds in treated soils, that of the corresponding seeds in untreated soils being* 100.

Treatment	Incubation time of							Temp. equiv.
	Wheat	Rye	Clover	Mustard	Rye grass	Fest. prat.	Mean	
	Harpenden Soil C, May 16							
Carb. disulph.	103	91	111	105	96	135	107	70°
Chloroform	91	117	93	145	121	110	113	81°
Benzene	89	109	104	105	131	105	107	70°
Ether.....	99	112	111	127	100	98	106	66°
Mean	*95*	*107*	*105*	*120*	*112*	*112*	*108*	*72°*
Mean of 1st 3...	—	—	—	—	—	—	*109*	*74°*
	Behaviour in this soil after heating							
Heated at 60°.....	84	140	103	94	95	108	104	—
,, ,, 80°.....	107	122	117	99	115	113	112	—
,, ,, 100°.....	112	165	157	107	134	130	138	—
	Harpenden Garden Soil, June 16							
Carb. disulph.	104	123	98	95	85	90	99	26°
Chloroform	130	93	99	129	125	98	112	60°
Benzene	97	129	187	131	139	95	130	65°
Mean	*110*	*115*	*128*	*118*	*116*	*95*	*114*	*61°*
	Ditto, July 20							
Carb. disulph.	105	146	112	92	114	102	112	60°
Chloroform	115	113	106	104	119	101	110	56°
Benzene	134	87	117	110	115	92	109	54°
Mean	*117*	*116*	*111*	*102*	*116*	*98*	*110*	*56°*
	Behaviour in this soil after heating							
Heated at 60°.....	131	106	105	135	129	87	112	—
,, ,, 100°.....	205	135	200	341	126	115	187	—

The relative times of incubation of six different sorts of seeds in these treated soils, as compared with the times of incubation in the two untreated samples, are given in Table II. Each determination was made in duplicate, and the details of the method of experimentation are the same as those already given in the previous communication (p. 415).

The mean results show that the treated soils in every case required a longer period of incubation than the untreated ones, and that the increase observed corresponds with that produced by heating to 74°, a very close agreement with the value, 66°, based on the determination of soluble matter. For the deduction of this temperature equivalent, the value obtained for these same seeds in heated soil has been taken from Table III of results previously published (p. 418), which were obtained with a similar, though not all with the same, soil sample: they are reproduced in the present table. The magnitude of the error in the incubation times is such that no weight can be attached to the differences in the values with the different antiseptics.

Although these results, in the writer's opinion, were sufficient to establish the fact that antiseptics produce a chemical change in soils, similar to that produced by heat, yet, differences depending on milligrams are often looked upon with suspicion, and it was, therefore, thought advisable to try and get differences of greater magnitude, by taking a soil very rich in organic matter.

This other sample of soil was from an old garden in Harpenden; it contained as much as 0·58 per cent. of nitrogen, though the amount of soluble matter was not much in excess of that in the first sample, ·093 against ·076.

The effect of ether on this soil was not examined, but three blank experiments were interpolated between the experiments with the other three antiseptics. The antiseptics were used in the proportion of 20 c.c. to 1 kilo. of soil, and the extracts were made by treating 100 grams with 1000 c.c. of water, 750 c.c. of the solution being evaporated; otherwise, the experiments were precisely similar to those already described. This sample of soil contained 23 per cent. of moisture, though it was in an apparently dry condition.

The results will be found in Tables I and II, those obtained immediately after the treatment of the soil being the ones dated June 16th. The increase in total soluble, and also in soluble organic matter, produced by the treatment, is much greater than in the case of the first soil, and amounts in the extreme case to 48 per cent. of that originally

present, thus placing the reality of this increase beyond question. The temperature equivalents were deduced from the determinations with heated soils given at the foot of the table, and the values for these equivalents are closely similar, whether the results are calculated from total soluble matter, or from the soluble organic matter only, the former leading to an average of 68° and the latter to one of 66°; and these are both practically identical with the value given by the first soil sample, namely, 69°. Moreover, with both samples of soil, the three antiseptics occupy the same order as regards their activity, carbon disulphide being decidedly more active than chloroform or benzene.

The germination results (Table II) afford further confirmatory evidence, the average incubation times of the seeds being appreciably greater than in untreated soil, and indicating, according to the values obtained with heated soils, as given at the foot of the table, a temperature equivalent of 61°. This is probably a little low, as the results with carbon disulphide seem to be exceptional, due no doubt merely to inevitable experimental error, as no such exceptional lowness is noticed in the results subsequently obtained on July 20th, which will be mentioned immediately. [The mean temperature equivalent is that corresponding with the mean of the percentage values in the preceding column: *e.g.* 61° is the temperature equivalent of a relative incubation period of 114. It should also be noted that, in plotting out the results with heated soil, the "unheated" soil is entered as having been heated to 30°, for reasons given in the previous paper, p. 420.]

Soils treated with antiseptics show an increased oxygen-absorption (Darbishire and Russell, *loc. cit.* p. 307) and, if this absorption affords a measure of the activity of the soil bacteria, it follows that partial sterilisation must increase the activity of, and, hence, the number of the surviving organisms (*ibid.* p. 305); and, according to Hiltner and Störmer, their number does eventually increase in soil treated with carbon disulphide, although the first action of the antiseptic is to reduce them by 75 per cent. If the increase in soluble matter in treated soil is due to this ultimate increase in bacterial activity, more soluble matter should be found after time has been allowed for the bacteria to multiply, than immediately after the treatment: the remaining values given in Tables I and II show, however, that the soluble matter diminishes with time.

The examination of the treated and untreated garden soils was repeated five weeks after the treatment, the soils (600 grams of each)

meanwhile having been left standing in the laboratory, side by side, in stoppered bottles of half-gallon capacity. In every case the total solids in the extract showed a marked diminution at the second examination, and so also did the soluble organic matter, except in the case of the sample treated with benzene, the average temperature equivalents having fallen from 68° to 61°, if the calculations are based on the total solids, or from 66° to 56°, if they are based on the organic matter. Whether the untreated soils have undergone any change during this time is doubtful, and if they have, it is certainly very small, and is confined to the inorganic matter, the organic constituents differing, on the average, by only ·0003 per cent. It will be noticed that the total solids in blank C give an appreciably higher value than in the other two blank experiments: the analysis was repeated on July 28th, and practically identical values were obtained; so this is not due to error.

The germination experiments (Table II) confirm the analytical results, the temperature equivalent of the incubation values having fallen in the five weeks from 61° to 56°.

An experiment, complementary to the above, was then made, in which the time available for bacterial action was reduced to the lowest possible limit. A sample of the same soil was treated with carbon disulphide for 10 minutes only; it was then exposed for 5 minutes, violently shaken with water for 15 minutes, and filtered during the next 30 minutes. The time during which the altered bacterial flora would be operating, would, therefore, be from 20 to 60 minutes. An untreated sample of soil was manipulated contemporaneously with this in exactly the same way. The treatment was found to have increased the total soluble matter by 31 per cent., and the soluble organic matter by 61 per cent., as against 23 and 48 per cent. respectively, after 18 hours, and 14 and 17 per cent. after 5 weeks. Clearly the increase in the soluble matter can not be due to progressive bacterial growth.

In all these experiments, the percentage of seeds germinating in the treated soils affords only negative evidence, because, in the case of these particular seeds, the germination percentage is not appreciably affected by small alterations in the character of the soil. Heating the soils to 60°—80° produces no result, and, as will be seen from the mean values entered in Table III, treatment with antiseptics is equally without effect.

That different antiseptics affect soils to different extents, is fairly evident from the composition of the extracts obtained, and that the

nature of the reaction is strictly identical in the various cases, is improbable; still more so, that it is strictly identical with that occurring when the soil is heated: but, that it is closely similar in all these cases, is evident from the fact that the temperature equivalent is practically the same, whether deduced from the effect on germination, or from the amount of soluble matter in the soils; in other words, the incubation period is increased *pari passu* with an increase of soluble matter, and as the effect is the same whether the soluble matter is increased by heating or by antiseptics, the nature of this soluble matter in both cases must be very similar.

TABLE III. *Mean percentage germination of seeds in soils treated with antiseptics, and in soils which have been heated.*

Treated with antiseptics				Heated	
	Harpenden Soil C				
Carb. disulph.	104	102		at 60°	106
Chloroform	99			at 80°	105
Benzene	101				
Ether	105				
	Garden Soil				
	June 16		July 20		
Carb. disulph.	107	99	100	at 60°	100
Chloroform	100		99	97 at 100°	87
Benzene	91		93		

The extent of the agreement as regards the temperature equivalent may be seen from the following summary, the maximum difference between the values based on the different data being only 6°.

Temperature equivalent based on				
	(*a*) Total extractive and (*b*) Organic extractive		Times of Incubation	Mean
Soil C	69°		74°	72°
Garden Soil	(*a*) 68°	67°	61°	64°
	(*b*) 66°			
Ditto, after keeping	(*a*) 61°	59°	56°	57°
	(*b*) 56°			

To these may be added the rough estimate based on Darbishire and Russell's experiments on plant growth, which gives 57°; this would apply to soils which had been kept for some time after treatment, but the closeness of its agreement with the above value for such soils is, of course, accidental.

In one case the action of paraffin oil was examined. The insecticidal properties of this, and the fact that both it and the semi-solid paraffins promote the decomposition of fruit when they are smeared over it, has led to the view that it must have a chemical action on various organic bodies, and it was of interest to ascertain whether confirmation of this could be obtained by finding that it acted on the organic matter in soil. A kilogram of the garden soil was, therefore, treated with 20 c.c. of a paraffin oil, b.p. 200°—250°, obtained from an American lighting oil, and it was found that after treatment the soil extract contained 0·2974 and 0·1384 per cent. of total and organic solids, respectively, these values showing an increase on those of the untreated soil, and representing a temperature equivalent of 74° and 70°, respectively; very similar to the equivalents obtained with other antiseptics. The results, however, are not strictly comparable with these, as the soil, after treatment, had to be exposed for three days before all traces of paraffin had evaporated.

As a conclusion from the present investigation, it would appear that the increased growth of plants in treated and in heated soils, alike, may be due solely to the chemical change induced by the treatment. Only one experiment appears at present to contradict this view: Darbishire and Russell (*loc. cit.* p. 318) grew three different crops in soil which had been heated to 90°—100°; watering them, in one case with sterilised water, and in the other with unsterilised water: the growth was much less vigorous in the latter case, which they attributed to the unsterilised water having disturbed the new bacterial flora which had been obtained by the heating. But the water used was well-water from a chalk district, and it was sterilised by heating it at 95°: this would materially alter its chemical composition, and quite possibly, therefore, alter its chemical action on the very sensitive compounds in the soil. Hence this experiment is inconclusive, and further evidence must be forthcoming before the intervention of bacterial action can be accepted as a factor in the matter.

Alteration in soils with time.

In the previous communication it was shown that very little change (as measured by the effect on germination) took place in heated soils when these were kept for some months in a dry condition at winter temperatures (p. 420); but that at high summer temperatures, and when in a moist condition, they lost, in a few days, part of their inhibitory effect (p. 426). A similar loss has just been seen to occur in soils treated with antiseptics, when kept for some weeks at ordinary summer temperatures in a fairly dry condition; and the loss of inhibitory properties in this case has been found to be accompanied by a diminution of soluble matter present, both inorganic and organic. The following experiments show that a similar diminution of, at any rate, soluble organic matter takes place under certain conditions in heated soils.

Glass pans, each containing 130 grams of the Harpenden soil C, which had been heated to various temperatures, were exposed to the air from May 28th to August 1st, being watered occasionally with distilled water, to simulate the conditions which would have obtained if crops had been grown in them. The water present varied from about 27 to 13 per cent. The pans were protected from the rain.

The composition of the extracts of these soils (180 grams with 1200 c.c. water) at the beginning and end of this period is given in Table IV. As regards the soluble inorganic matter, no definite change seems to have occurred, the values being mostly very small, and the signs of them distributed irregularly; but, as regards the organic matter, the case is very different, the soils which contained the smaller proportion to start with having increased their stock very considerably, whilst the highly heated soils, which contained a large proportion at first, have had this considerably diminished, these two opposite changes neutralising each other with soil heated to 80°[1].

That the matter rendered soluble by heating a soil becomes insoluble again in the presence of water, is also evidenced by the fact that the extract of such a soil becomes cloudy after standing for a few days. This occurs even when it contains 0·5 per cent. of mercuric chloride, which would prevent any bacterial action.

The co-existence of two opposite changes in heated soils must,

[1] The values for the percentage alteration in the last column all lie on an appreciably straight line, except that with the unheated soil; how far this really implies an intrinsic difference in behaviour between this and the heated soils, it is impossible to say.

naturally, complicate the question considerably, and renders it improbable that the real effect of heating a soil on the growth of plants in it, will be settled by experiments made with soil heated to one temperature only, especially when the temperature usually selected is in the neighbourhood of that where the changes counteract each other.

TABLE IV. *Alteration in soluble matter in soil by exposure and moistening.*

Soil Heated to	Soluble inorganic			Soluble organic		
	May 28	Aug. 1	Alteration	May 28	Aug. 1	Alteration
30°	·1002	·0920	·0082 = − 8 p.c.	·0556	·0907	+ ·0351 = + 63 p.c.
60°	·0777	·0827	+ ·0052 = + 7 ,,	·0638	·0716	+ ·0078 = + 12 ,,
80°	·0786	·0785	− ·0001 = 0	·0743	·0745	+ ·0002 = 0
100°	·0767	·0854	+ ·0087 = + 11 ,,	·1073	·0934	− ·0139 = − 13 ,,
125°	·0976	·1022	+ ·0046 = + 5 ,,	·2279	·1750	− ·0529 = − 23 ,,
150°	·1276	·1132	− ·0144 = − 11 ,,	·3929	·2493	− ·1436 = − 37 ,,
Average...	—	—	− ·0007, + 0·7 ,,	—	—	—

The rate at which these changes occur must, doubtless, be influenced largely by the minutiæ of the circumstances of the experiments, such as the temperature, the degree of the moisture, access of air and carbon dioxide, possibly, also, by that of light, and by the fluctuation in these conditions. This complexity will no doubt explain certain apparent discrepancies in the results obtained. Darbishire and Russell state that no effect produced by the heating of the soil was observable on germination in their experiments wherein seeds were sown in the ordinary way in pots of soil exposed to the open air (*loc. cit.* p. 322: and *Nature*, July 4, 1907), and a similar absence of effect has been recently found by the present writer under similar conditions, even with soils heated to 150°: this can only be explained by supposing that these conditions have destroyed the inhibitory substance, whereas the more rapid germination, coupled with a practical absence of ventilation, in sporulating dishes in an incubator, obviated such changes.

Several series of preliminary experiments have been made on the growth of plants in soil heated to various temperatures, under circumstances somewhat different from those obtaining in Darbishire and Russell's experiments, and the results indicate that the nature of these circumstances exercises a considerable influence in this matter too.

It certainly appears evident that growth in soils heated to temperatures of 125° and 150° is generally more feeble than in soils more moderately heated, and that increased growth with increased heating of the soil is not an invariable rule. It may be noted that both in Darbishire and Russell's experiments with plants, and in my own with apple trees, the results in highly heated soil were compared with those in soil heated to only 80°, not to 100°. The argument against the view that growth is dependent on bacterial conditions—based on the supposed fact that this growth increased with the heating of the soil up to temperatures at which all bacteria are killed, and the oxygen absorption is reduced to a minimum—must, therefore, lose its force; but it does not thereby become converted into an argument against the chemical explanation of the phenomena, for it is probable that at and above about 125° heating produces chemical changes different in nature from those at lower temperatures: the figures representing the effect of the temperature of heating on the percentage germination and times of incubation of seeds (*loc. cit.* p. 419) both indicated a "break" at this point, and it has been repeatedly noticed, during the evaporation of the soil extracts, that those obtained from highly heated soils were evidently of a different character from the others.

Examination of various soils.

The behaviour of soils of different origin towards germination, as well as the alteration produced in them by heat, was partially examined in the previous work, but the number of instances taken—only three, in some respects four, soils—was too small to permit of any very general conclusions, and the comparative behaviour of these soils and of pure water was left in a state of uncertainty, owing to the seeds in the water having been germinated on moist blotting paper, which may not be analogous in its action to moist soil.

This work has now been extended with other soils, and the water cultures were made in specially purified precipitated silica.

The soils selected were chiefly those which were exceptionally poor or exceptionally rich, as measured by their behaviour under ordinary cultivation. The total nitrogen in the dry soils, and the percentages of soluble matter in them before and after heating, are given in Table V, and their total water capacity in Table VI. The latter was determined by saturating the soil, allowing it to drain, and then drying it at 100°, the results being expressed as the weights of water absorbed by every

100 parts of the soil dried in this way. After heating at 100°, the water capacity, as will be seen, is considerably reduced, and there is generally a slight further reduction after heating at 150°. The hygroscopic capacity of some of these soils after heating at 100° (determined by exposing them to moist air till constant) is also given in the table.

TABLE V. *Percentage of nitrogen and of soluble matter in various soils.*

Soil	N	Soluble matter			
		Unheated		Heated at 150°	
		Total	Organic	Total	Organic
P. Takoma	·0659	·0600	·0244	·2529	·2076
P. Millbrook	·0720	·0805	·0262	·2358	·1561
P. Poor pasture	·2252	·0841	·0394	·4006	·3137
R. Hop garden	·1894	·0807	·0398	·4075	·3238
P. Exhausted	·104	·1029	·0403	·2521	·1587
R. Tawton	·1871	·0797	·0430	·4023	·3241
R. Plymstock	·3180	·1104	·0561	·4378	·2599
R. Best pasture	·3839	·0953	·0576	·8976	·7749
Wye	·17	·1275	·0583	·3491	·2542
R. Garden	·58	·2303	·0899	1·1378	·8615
Harpenden C.	·25	·2027	·0987	·4483	·3272

TABLE VI. *Water capacity of soils.*

Soil	100 parts of dry soil absorb			Heated at 100° and exposed to moist air
	Unheated	Heated at 100°	Heated at 150°	
Silica	—	—	313·4	26·2
Takoma	37·8*	31·0*	24·8*	5·1
Tawton	47·6*	45·6*	42·4*	—
Millbrook	35·5*	29·8*	27·3*	2·3
Hop garden	51·4*	42·7*	40·2*	—
Poor pasture	56·4*	54·2*	46·5*	—
Best pasture	74·9*	53·8*	53·4*	—
Exhausted	52·7	36·1	—	5·4
Plymstock	80·3*	61·8*	65·6*	—
Wye	47·9	—	—	—
Harpenden C.	53·5	—	42·7	17·3
Garden	92·6	75·3	—	—
Mean of those marked *	*54·9*	*45·6*	*42·9*	—

The Takoma lawn soil was supplied to me through the courtesy of Mr Milton Whitney, of the American Bureau of Soils. It is a soil which has formed the subject matter of much investigation by the Bureau, and has been found to be exceptionally unfavourable to plant growth (see especially Bulletin No. 28; also No. 23, p. 46). It is, certainly, a very peculiar soil: it is a light, sandy loam, which contains less soluble matter, either total or organic, than any of those here examined, and it yields a very light coloured extract: yet it contains the comparatively large quantity of 3 per cent. of total organic matter: after heating, it appears to become quite poisonous, emitting an offensive odour, and causing seeds sown in it to become black.

The soil from Millbrook is from derelict land on the Greensand in Bedfordshire. This particular sample was drawn from a plot which has received a moderate dressing of general artificial manures during the past eight years. The Exhausted soil was some from the Barnfield at Rothamsted, which has received no manure, and has been continually cropped with roots since 1843. The Hop garden, the Poor pasture and Best pasture soil, were soils from Ockham, Sussex, all from the same geological formation. Those from Plymstock and North Tawton were both arable soils, selected as being amongst the most fertile in Devonshire. The latter was a sample of the well-known red soil; the former, also, was reddish in colour, but came from the limestone, not the red sandstone, district. The Plymstock soil had not been manured for 18 months. The Harpenden soil C, and the Garden soil, have already been mentioned above, and that from Wye, Kent, is the same sample as that used in the previous experiments. The composition of the extracts of the Harpenden and Wye soils differs from that given previously, partially owing to the changes which have occurred in the soils on being kept, and partially owing to the different method of extraction employed: but the former and present determinations lead to similar relative values.

In Table V the poor and rich soils are marked P and R, respectively, the Wye and Harpenden soils being fairly rich. They are arranged in their order as regards the soluble organic matter in them before heating, and this, as will be seen, agrees fairly well with an arrangement based on their productiveness, the Hop garden soil being the most conspicuous exception. The amount of soluble organic matter present after heating bears no evident relation to that present in the unheated soil.

In conducting the experiments, a certain number of the soils were heated at the same time in sealed flasks in the autoclave. The soluble

matter was determined immediately afterwards, and the germination experiments started. In these, soils heated to 100° were used, as well as those heated to 150° and the unheated soils. The determinations had to be made in three separate series, and each series included similar determinations in which silica was used. The silica experiments were made in triplicate, and those with soils in duplicate.

TABLE VII. *Percentage germination of seeds. The values for silica are actual percentages, those for the soils are the values compared with silica as* 100.

Soil	Germination of						
	Wheat	Rye	Clover	Mustard	Rye grass	Fest. prat.	Mean
	First Series						
Silica	97	70	100	83	47	73	--
Takoma	103	107	90	120	128	103	108
Millbrook	88	86	100	120	74	110	94
Garden	102	79	100	114	96	123	102
Exhausted	103	100	90	120	106	123	107
	Second Series						
Silica	90	50	97	97	53	77	--
Best pasture	100	70	98	98	85	91	90 (94)*
Poor pasture	106	60	93	98	75	110	90 (96)*
Hop garden	100	20	103	93	85	117	86 (100)*
Plymstock	83	100	93	88	85	91	90 (88)*
Harpenden C	106	30	103	103	58	110	85 (96)*
Wye	106	50	103	88	113	117	96 (105)*
	Third Series						
Silica	85	55	100	100	35	85	—
Tawton	106	73	95	95	100	100	93

* Values with Rye omitted.

The soils during heating contained one-sixth of the maximum water retainable by them, and, for the germination experiments, water, previously digested with precipitated silica, was added, so as to raise the contents to five-sixths of the maximum. With silica itself the water had to be raised to nine-tenths of the maximum: with a lesser quantity the silica did not become wetted throughout.

The percentages of the seeds germinating in the unheated soils, as compared with those in silica, are given in Table VII, and the average

times of incubation in Table VIII. As regards the former, the values with Rye in the second series are nearly all exceptionally low, and it seems preferable to omit them, and to take those mean values which are given in brackets in the last column. The germination percentages are always subject to accidental errors, and much less weight can be attached to them than to the incubation times.

TABLE VIII. *Incubation times of seeds. Actual (in days) in the case of silica. Relative (compared with that in silica as* 100*) in the case of soils.*

Soil	Incubation time of						
	Wheat	Rye	Clover	Mustard	Rye grass	Fest. prat.	Mean
	First Series						
Silica	1·44	1·33	1·00	1·07	4·21	3·55	—
Takoma	113	109	120	93	80	105	103
Millbrook	130	88	105	106	69	88	103
Garden	99	141	115	118	97	104	112
Exhausted	135	129	128	150	99	95	123
	Second Series						
Silica	1·38	1·33	1·02	1·15	4·13	3·51	—
Best pasture	136	106	154	128	119	73	119
Poor pasture	129	113	130	115	81	82	108
Hop garden	108	75	98	100	80	78	90
Plymstock	115	126	127	119	121	69	118
Harpenden C	129	75	98	100	85	99	98
Wye	119	85	118	97	76	90	97
	Third Series						
Silica	2·42	2·39	1·20	1·40	2·88	3·09	—
Tawton	72	84	110	132	97	102	99
	Clover	Mustard	Wheat	Rye	Rye grass	Fest. prat.	
Mean actual time (Silica)	1·07	1·21	1·75	1·68	3·31	3·38	—
Mean relative time (Soils)	118	117	117	103	91	90	—

The mean results have been set out in Table IX, entering the soils in their order in respect to soluble organic matter. That these soils are in general less favourable to germination than silica is, seems evident, the period of incubation being greater (by 6 per cent.), and the percentage of seeds germinating being slightly less. The lengthening of the

incubation period is also apparent, when the seeds germinating within a given time (one or two days, according to the different seeds) are considered, the mean values (fourth column) as compared with silica showing a deficiency of 12 per cent. That the difference in the values with individual soils is not due to experimental error (at any rate in the times of incubation), is fairly evident on examining the detailed results in Tables VII and VIII; but, whether there is any definite connexion between the values obtained and the soluble organic matter in the soils, is very doubtful. On taking the means of the first six and last six entries, the latter, or richer soils, appear on the whole, and from every point of view, to be the least favourable for germination, but the differences are too small, and the irregularities too great, to admit of this being accepted as a rule.

It is doubtful whether any of these soils act more favourably towards germination than does pure water. The only one which gives a value appreciably less than 100 for the incubation times is the Hop garden soil, and that value is not supported by the percentage germination or the number of seeds germinating in a given time. The conclusion, therefore, drawn from the earlier experiments—that some soils, and especially those rich in soluble organic matter, are more favourable than is water towards germination—though correct, perhaps, so far as the particular instances examined were concerned, is not correct in any general sense.

On looking at the values entered in the last two lines of Table VIII it will be seen that, with the two grasses, the average incubation times are less in the soils than in silica, instead of being greater, as with the other seeds. This is probably not due directly to the different nature of the seeds, but to changes occurring in the soils before the seeds germinated. It was shown in the previous communication that the inhibitory substance present in heated soils is partially destroyed by digestion for a few days in the presence of water in the incubator, and the values in Table VIII show that the seeds which give these low results are precisely the ones which take a long time to germinate; indeed, with one irregularity in the case of Wheat or Rye, the increase in the relative incubation times is inversely as the actual length of that time. By taking the four seeds only where the soil has had less time to be modified before germination commences, the values for the increase in the incubation times become accentuated, the mean being raised to 114, as shown by the values given in the last column of Table IX: no doubt, if still more rapidly germinating seeds could be obtained, the inhibitory property of the soils would be still more marked.

It will be noticed that, with the grasses, the soils not only do not act in an inhibitory manner towards germination, but actually favour it, (the incubation times being less than 100); if, as is suggested, this is due to the changes produced in the soil by digestion in the incubator, we may well expect to find some soils under certain natural conditions acting in a like favourable manner. It is interesting to note, also, that the comparative values obtained with different soils are closely similar, whether the grass seeds are included or omitted (cols. 3 and 6 in Table IX).

TABLE IX. *Germination of six kinds of seeds in various soils. Values compared with those in silica as* 100.

Soil	Per cent. soluble organic matter	Incubation time		Seeds germinating in a given time		Percentage germination		Incubation time, four seeds only	
P. Takoma	·0244	103		100		108		109	
P. Millbrook ...	·0262	103		93		94		107	
P. Poor pasture	·0394	108	104	79	87	96	100	127	109
R. Hop garden	·0398	90		90		100		95	
P. Exhausted ...	·0403	123		74		107		136	
R. Tawton	·0430	99		86		93		100	
R. Plymstock ...	·0561	113		87		90		122	
R. Best pasture	·0576	119		76		94		131	
Wye	·0583	97	106	100	88	105	97	105	110
R. Garden	·0899	112		89		102		118	
Harpenden C	·0987	98		89		96		101	
Mean	—	*106*		*88*		*99*		*114*	

The results with these same soils after heating are given in Tables X and XI, the values being the relative values when compared with the individual soil in an unheated condition. With the Takoma and Best pasture soils, the water added remained partially unabsorbed, even at the end of the 12—14 days throughout which the germination extended. With the Pasture soil a repetition was made, stirring the soil and water together before sowing the seeds, the results thus obtained are recorded in the second lines of values entered against this soil: they differ very little from the first set of values. With the Poor pasture and Hop garden soils, the values for the relative percentage germination with Rye have been omitted in the means, as they are very uncertain, owing to the smallness of the actual number of seeds germinating in the unheated soils.

TABLE X. *Actual percentage germination of seeds in unheated soils, and relative germination in similar heated soils, when that in the unheated soil is represented by* 100.

Soil	Germination of						
	Wheat	Rye	Clover	Mustard	Rye grass	Fest. prat.	Mean
Takoma,	[100	75	90	100	60	75]	100
,, 100°	100	67	106	100	67	107	75
,, 150°	85	47	39	35	8	120	57
Millbrook,	[85	60	100	100	35	80]	100
,, 100°	118	125	100	100	83	94	103
,, 150°	118	133	95	100	100	111	110
Garden,	[95	55	100	95	45	90]	100
,, 100°	105	118	100	100	122	56	100
,, 150°	95	73	95	79	33	72	74
Exhausted,	[100	70	90	100	50	90]	100
,, 100°	100	129	111	95	70	89	99
,, 150°	100	107	111	100	80	72	95
Best pasture,	[90	35	95	95	45	70]	100
,, ,, 100°	83	183	105	100	78	121	112 111
	110	143	100	100	78	129	110
,, ,, 150°	94	100	95	89	67	136	97 92
	89	86	74	84	67	114	86
Poor pasture,	[95	30	90	95	40	85]	100
,, ,, 100°	100	(200)	106	105	125	94	106
,, ,, 150°	100	(217)	100	105	88	94	97
Hop garden,	[90	10	100	90	45	90]	100
,, ,, 100°	111	(450)	100	111	111	106	108
,, ,, 150°	111	(100)	90	111	78	111	100
Plymstock,	[75	50	90	85	45	70]	100
,, 100°	127	130	111	118	56	79	103
,, 150°	133	70	106	112	67	121	101
Tawton,	[90	40	95	95	35	85]	100
,, 100°	100	113	105	105	43	88	92
,, 150°	100	113	89	95	71	76	91

The mean results are set out in Table XII, and show that the greater the increase in soluble organic matter produced by heating, the greater is the inhibitory effect of the soil on germination, the times of incubation being increased, and the percentage of seeds germinating being decreased; the action in both cases is far more marked with soils heated to 150° than 100°. All these soils, therefore, behave in the same way as those previously investigated: but there is clearly no very strict connexion between the increase in soluble matter and the increase in the inhibitory effect. The connexion becomes still less exact if the heated soils are compared as regards the actual amounts of soluble

organic matter in them, instead of as regards the increase of such matter produced by the heating: this is shown in the lower part of the Table, the incubation results in the most highly heated soils being alone entered here. The Takoma soil, in this case, evidently behaves in an exceptional manner. The other arrangement entered in this part of the Table sets the results out in reference to the organic matter

TABLE XI. *Incubation times of seeds. Actual (in days) in the case of unheated soils: relative (as compared with that in the unheated soil represented by* 100) *in the case of the heated soils.*

Soil	Incubation time of						
	Wheat	Rye	Clover	Mustard	Rye grass	Fest. prat.	Mean
Takoma,	[1·63	1·44	1·20	1·00	3·37	3·72]	100
,, 100°	126	162	113	113	113	104	122
,, 150°	177	356	208	540	267	157	284
Millbrook,	[1·87	1·17	1·05	1·45	2·92	3·13]	100
,, 100°	119	110	138	148	152	134	133
,, 150°	114	170	200	169	129	122	151
Garden,	[1·42	1·87	1·15	1·26	4·08	3·68]	100
,, 100°	158	85	161	164	113	136	136
,, 150°	193	120	291	552	89	139	231
Exhausted,	[1·95	1·72	1·28	1·60	4·17	3·37]	100
,, 100°	103	102	137	109	102	88	109
,, 150°	114	108	173	156	78	111	121
Best pasture,	[1·87	1·42	1·57	1·47	4·93	2·57]	100
,, ,, 100°	(100	235	89	107	105	170	142 | 141
	(130	164	179	150	91	124	140
,, ,, 150°	(147	337	379	355	151	210	261 | 276
	(154	305	427	519	139	194	290
Poor pasture,	[1·69	1·50	1·33	1·32	3·33	2·89]	100
,, ,, 100°	88	103	95	123	164	98	112
,, ,, 150°	109	113	110	197	129	94	125
Hop garden,	[1·49	1·00	1·00	1·15	3·30	2·75]	100
,, ,, 100°	154	188	120	130	117	106	136
,, ,, 150°	127	260	463	322	189	146	250
Plymstock,	[1·58	1·67	1·29	1·37	5·00	2·40]	100
,, 100°	146	120	128	171	115	152	139
,, 150°	116	237	214	231	73	184	179
Tawton,	[1·75	2·00	1·32	1·85	2·78	3·16]	100
,, 100°	117	84	114	119	99	91	104
,, 150°	143	99	274	155	156	125	159

originally present in the unheated soils, and although, on the whole, the soils which were richer in this respect become the most inhibitory towards germination after heating, yet the instances which are exceptional are too numerous to admit of this being accepted as a general proposition.

TABLE XII. *Increase in incubation periods and percentage of germination, compared with the soluble organic matter in the heated and unheated soils.*

Soil	Per cent. increase in organic matter at 150°	Per cent. increase in incubation time			Per cent. difference in germination			
		at 150°		at 100°	at 150°		at 100°	
P. Exhausted ...	295	21		9	- 5		+ 1	
R. Plymstock.....	363	79		13	+ 1		+ 3	
P. Millbrook	496	51	47	33	+ 10	- 1	+ 3	+1
R. Tawton	654	59		4	- 9		- 8	
P. Poor pasture	696	25		12	- 3		+ 6	
R. Hop garden ...	713	150		36	0		+ 8	
P. Takoma.....	751	184	160	22	- 43	19	- 25	- 2
R. Garden	857	131		36	- 26		0	
R. Best pasture...	1245	176		41	- 8		+ 11	
Mean	—	*97*		*23*	*- 9*		*- 5*	

Soil	Organic matter at 150°	Per cent. increase in incub. time		Soil	Organic matter in unheated	Per cent. increase in incub. time	
Millbrook	·1561	51		Takoma	·0244	184	
Exhausted	·1587	21		Millbrook	·0262	51	
Takoma	·2076	184	72	Poor pasture ...	·0394	25	86
Plymstock	·2599	79		Hop garden ...	·0398	150	
Poor pasture ...	·3137	25		Exhausted	·0403	21	
Hop garden ...	·3238	150		Tawton	·0430	59	
Tawton	·3241	59	129	Plymstock	·0561	79	111
Best pasture ...	·7749	176		Best pasture ...	·0576	176	
Garden	·8615	131		Garden	·0899	131	

The poverty or richness of a soil under ordinary cultivation bears no relation to its behaviour when heated. This is best seen from the list in the upper part of the table, for there the poor and rich soils figure alternately. There also appears to be no direct connexion between the total nitrogen in the soil (as given in Table V) and the extent to which it is altered by heat.

The general conclusion from these results is either that the inhibitory substance produced by heating different soils is not the same in all cases, or, more probably, that it constitutes only one of the organic products formed when a soil is heated, the proportions between it and the other non-inhibitory substances formed varying in each particular case.

Summary.

When soils are treated with antiseptics, such as carbon disulphide, chloroform, benzene, ether or paraffin oil, they undergo chemical change, and the soluble organic matter in them is increased, just as in case of their being heated; they also exhibit the same inhibitory effect on the germination of seeds that heated soils do.

The different antiseptics differ in the intensity of their action, but the inhibitory substance formed is probably the same in all cases, and also the same as that formed by heat, for the quantity formed has the same effect on seeds, whether produced by antiseptics or by heat.

On keeping treated soils for a few weeks at a summer temperature, some of the organic matter which was rendered soluble becomes insoluble, and the inhibitory action is reduced. This is also the case with heated soils, especially when repeatedly watered; though with unheated soils under similar conditions the soluble organic matter increases.

The treatment of soils with antiseptics induces a change equivalent to that obtained by heating the soil to 60°—75°, and this may be sufficient to account for the increased growth observed in plants grown in them.

The production by heat of a substance inhibitory to germination appears to be a property common to all soils, twelve instances having been examined: the proportion of it formed depends on the increase in the amount of organic matter rendered soluble by heating; but the actual amount of the soluble organic matter in the heated soil is not always a criterion as to the intensity of its inhibitory action, and still less is the amount of soluble organic matter originally present in the unheated soil, though in the majority of cases it may be so. There appears to be no connexion between the fertility of a soil and the extent to which it is altered by heating.

Soils in their natural state appear generally to contain a certain amount of this inhibitory substance, as they act less favourably towards germination than pure water does: whether in any cases soils can act more favourably than water—as the earlier experiments had indicated they could—is open to doubt, but the probability is in favour of their doing so. So far as the instances now examined are concerned, the richer soils, and those containing most soluble organic matter, are slightly less favourable to germination than the poorer soils.

THE YEAST FLORA OF BOTTLED CIDERS.

By ELSIE B. PEARCE, B.Sc.
Assistant Lecturer in Botany, University College, Bristol;
AND
B. T. P. BARKER, M.A.
Reader in Economic Biology, University College, Bristol,
National Fruit and Cider Institute, Long Ashton, Bristol.

Introductory.

THE work described in this paper deals exclusively with the yeast flora of certain bottled ciders. It was undertaken as a section of an extensive scheme of investigation of the organisms which are concerned in the fermentation of English-made ciders. Apart from the more purely biological side of the question the main objects of the whole scheme are to determine how far the customary method of conducting the fermentation of cider in this country is satisfactory, and whether it could be improved, from a practical point of view, by certain modifications or by the substitution of other processes, such, for example, as the use of selected yeasts. Fermentation with pure cultures of special yeasts has been adopted with great success in the brewing industry (Jörgensen, 8), owing mainly to the extensive researches of Hansen and other workers, following similar lines to those of the Danish biologist. It has also been applied with some success to the preparation of wines by Wortmann (10) and others. Its use in connexion with cider has not been so exhaustively tested, although Jacquemin (7), Lloyd (9), Allwood (1), and Barker (3) among others have published records of experimental work upon the subject.

The usual method in cider making is to allow the freshly pressed apple juice to ferment spontaneously; and therefore the organisms responsible for the fermentation mainly owe their presence in the juice

to their occurrence either on the fruit itself at the time of grinding or on the surface of the machinery, casks, and other appliances with which the juice comes in contact. Some may, and probably do, find their way into the juice from the air of the cider factory, but it is doubtful if in the average case these exert much influence on the nature of the fermentation, since their number must be very small as compared with those derived from the other sources mentioned. Very little appears to be known of the characters of the organisms found in the juice or mature cider. Kayser (6) and Dienert (4) have, however, described the characters of several different varieties which were isolated from French ciders. It is probable, judging from the results which have been obtained thus far in the course of our work on the whole subject which will be published in due course, that the yeast flora varies considerably, both in the fermenting juice and the mature product. On account of the great diversity in character of most English ciders it is of importance to ascertain to what degree the nature of the flora is responsible for the difference; and in addition to determine whether the character of the flora depends upon certain conditions or is purely fortuitous. The former question will be discussed in a later article, the present paper being concerned with the latter question.

The most obvious of the factors which may possibly affect the character of the flora are the variety or varieties of apples from which the cider is made, the district in which the fruit is grown, the nature of the season,—all of which might have some determining influence as to the kinds of yeast which find their way into the juice from the fruit itself,—and the factory where the cider is made. The latter factor is brought into the question on account of the organisms which find their way into the juice from the appliances used in cider making, since it is conceivable that certain characteristic sets or combinations of organisms might regularly occur in different cider houses. Each of the factors mentioned has been to some extent involved in the work dealt with in this paper.

The plan adopted was the examination of the flora of five samples of bottled cider, made in each case at the National Fruit and Cider Institute. Three of the samples were made from Sweet Alford apples only, one being made in the autumn of 1904, another in the autumn of 1905, and the third in the autumn of 1906. Each lot of fruit concerned was grown in Devon, but in different districts of the county. The other two samples were made from Kingston Black

apples only, one in the autumn of 1904, and the other in the autumn of 1906. The former was obtained from Devon fruit, and the latter from Somerset fruit. In every instance the ciders were bottled in the spring of the year following the time of making, and they remained in the bottles untouched until last July, when the examination of their respective floras was made. Each cider was filtered perfectly clear before bottling, but an appreciable deposit, consisting largely of yeast cells, was formed gradually.

Methods of isolation and purification.

The method used to isolate in the pure state the different varieties of yeast present in the ciders under investigation was that of fractional plate cultures on 10 °/₀ beer-wort gelatine. The procedure was as follows:—

In the case of each sample, tubes of sterilised beer-wort were infected with a small quantity of the cider (the bottle having been previously well shaken), and were incubated for 24 hours at 27° C. in order to obtain the cells in an active condition.

At the end of this time a tube of 10 °/₀ beer-wort gelatine was infected with a drop of the liquid and a series of seven fractional plate cultures was made.

Subsequently the plates were examined, and the various species present determined broadly and isolated, after an examination of the external characters of the various colonies and the microscopic appearance of the vegetation.

In plates with comparatively few colonies it could be assumed with a fair amount of probability that each was the result of a growth from one single cell. From such well-separated colonies streak cultures were then made on tubes of sloped beer-wort gelatine and the growth obtained might be regarded as probably a pure culture.

The purity of the streaks was then tested by making a fractional series of four plate cultures from each. The colonies obtained were carefully examined both as regards external morphology and vegetation to see if any difference could be detected between them. If all the colonies were identical (which was found to be so with one exception), a fresh "repurified" streak was made on beer-wort gelatine from a single colony and kept as a stock tube. In the case of the impure culture the streak kept as a stock was made from a well-isolated colony showing the typical form. Each time the stock tube was opened to obtain a sample

of the growth, a new streak was made, to prevent the cultures becoming impure through infection from the air. Also plate cultures were made occasionally to see if the yeast kept true to the type, and a new streak was made from a colony on one of the plates and used as a stock tube.

In this way some fifteen different varieties were obtained and their behaviour then studied under varying cultural conditions.

Cultural methods.

The results obtained in the culture of the yeasts upon different media were used both from the point of view of ascertaining the specific characters of each form, and also of distinguishing the varieties from one another.

The characters which proved of greatest value for this purpose were as follows:—

(i) *Budding.*

The method of budding was studied in hanging-drop cultures of 10 % beer-wort gelatine, the development of a single cell being kept under microscopic observation for several generations.

Some varieties exhibited a very characteristic type of growth, but in general the differences were not sufficiently well marked to make this a reliable character upon which to base any distinction of species. This may be seen by reference to the figures.

(ii) *Form of colonies on plate cultures.*

The colonies in most cases show a more or less characteristic appearance, which was found very convenient in separating the varieties in the first instance.

Some were dry and solid-looking, while others were moist and semi-transparent. The margins of the colonies also differed, some being smooth, while others were irregular, crenate or somewhat mycelioid.

(iii) *Streak cultures.*

The growth which occurs shows very much the same characters as the colonies on plate cultures, but this method has the additional advantage that the development can be watched over a longer period, as a streak is more easily kept pure and also dries up less quickly.

Streak cultures were also made and compared, to determine as far as possible the relative rates of growth, the comparison always being made with streaks of the same age.

The characters found most convenient for distinguishing between the different forms were :—

1. The general form of the growth, *i.e.* whether compact or spreading, dry or moist.

2. The nature of the growth, whether flat or heaped up on the gelatine.

3. Characters of the slopes of the streak, *i.e.* whether smooth or corrugated.

4. Characters of the margins, *i.e.* whether smooth, crenate, irregular or mycelioid.

The extreme similarity of some of the streaks obtained first gave the suggestion that some of the varieties were identical—a suggestion which in two cases subsequently proved to be correct.

(iv) *Form of vegetation.*

Although in itself somewhat unreliable evidence of the identity or otherwise of yeasts, yet with due precautions it may yield valuable information.

Comparative observations were made of the vegetation of the yeasts under exactly similar conditions, *e.g.* :—

1. Young cultures in beer-wort incubated for 24 hours at 26° C.

2. Sedimentary vegetation in beer-wort cultures when fermentation had finished, *i.e.* after about 15 days incubation at 26° C.

3. Young cultures on beer-wort gelatine, either on streaks or plates.

4. Forms of cells in old streak cultures on beer-wort gelatine, about 4 months old. The cells were generally found to vary considerably in size and shape, and the protoplasmic contents to assume a characteristic appearance.

The sizes of the cells were also determined, but were found to be exceedingly variable, and, compared with ordinary beer yeasts, very small.

(v) *Films.*

Flasks containing a quantity of beer-wort were infected with each yeast, and allowed to remain undisturbed for some time to ascertain if films were formed. No satisfactory results were obtained, as with three exceptions no definite films occurred. In some cases a very slight film formation was observed, similar to that often observed on liquids containing bacteria.

This character, therefore, was not of much use for our purpose in most instances.

In a few cases a well-defined yeast ring was formed.

(vi) *Spore formation.*

Various methods were used to try and obtain an abundant supply of spores, but none were very successful.

1. The ordinary plaster block method, *i.e.* a few drops of a young vigorous culture in beer-wort which had been incubated for 24 hours at 26° C., were poured over the block, which was kept in a covered dish and moistened with sterile distilled water.

2. Pieces of sterilised porous porcelain in Petri dishes were substituted for the blocks, and infected both with beer-wort cultures, and also with cultures in glucose-Mayer solution, the latter however yielding poor results. The cultures were kept at various temperatures in the hope of determining the optimum temperature for spore formation, but the results obtained were so exceedingly variable as to be valueless for this purpose.

3. Plates were also kept in closed air-tight vessels, in the presence of strong caustic potash solution to absorb any CO_2 which might be formed, as it was thought that an accumulation of the gas might hinder the formation of spores; but except in one case there was no improvement.

4. Another attempt was made by cultivating the yeasts on sterilised blocks of carrot and potato in tubes, incubated at 26° C., and in most cases this proved the most satisfactory method.

The growths were examined at intervals and the first indications of the formation of spores noted.

Since each method failed to give spores in the case of some varieties, it must be concluded that these are Torula forms which do not possess the habit of forming spores.

Another point which added considerably to the difficulties was that apparently the yeasts gradually lost the property of forming spores after prolonged cultivation on artificial media. In the later part of the work a much longer time was needed before any appearance of spore formation occurred, and even then the percentage of cells which contained spores was much smaller.

(vii) *Germination of spores.*

The best method for observing stages in the germination of the spores was found to be that of infecting tubes containing a very small quantity of beer-wort with a comparatively large amount of a culture containing spores.

The liquid was heated to 60° C. for 5 minutes, by which treatment all the vegetative cells were generally killed, and after 24 hours incubation at 26° C., a sample of the culture was examined.

Conclusive proof was obtained that the bodies within the cells were true spores, as the mother-cells were seen in many cases to have burst, and the swollen discharged spores to have commenced budding, while in other instances various stages of the swelling of the spores and the disruption of the mother-cell wall, leading up to the liberation of the spores, were noted.

(viii) *Temperature limits of growth.*

The maximum temperatures for the different varieties were determined by observing the amount of growth of streak cultures on 2 % beer-wort agar.

The range of temperature was found to be fairly wide, one form flourishing at 38° C., although most seemed to group themselves about the region of 35° C. or just above or below that temperature.

The temperatures tested were 28° C., 30° C., 32·5° C., 35° C. and 38° C.

The minimum temperature limits were not ascertained, each form being capable of growth below 6° C.

(ix) *Action on sugars.*

The fermentative power of the yeasts was tested by infecting tubes of Mayer solution[1] with each variety, the sugars used being dextrose, laevulose, saccharose, maltose and lactose respectively.

It was found that in many instances the fermentations were practically identical, and in all cases lactose remained untouched.

Two forms gave well-marked films on all the liquids and one other produced a film on maltose only. In these cases no fermentation was induced in any of the sugars.

(x) *Liquefaction of gelatine.*

Several of the forms isolated possess the property of liquefying solid gelatine media, some after about a fortnight, others only in fairly old streak cultures. The vegetation in the liquid gelatine generally seems to assume rather abnormal characters, perhaps owing to lack of oxygen.

Other forms cause a very slight amount of liquefaction,—especially noticeable in hanging-drop culture, where the gelatine seems to become liquid just in the immediate vicinity of the cells. Probably all those varieties which have a moist streak possess this property to a small extent, although not markedly enough to cause the whole of the gelatine to become liquid.

[1] Formula used: ammonium tartrate 1 grm., KH_2PO_4 ·5 grm., $MgSO_4$ ·5 grm., $Ca_3(PO_4)_2$ ·05 grm., sugar 10 grms., water 100 c.c.

Detailed description of the species isolated.

From Sweet Alford Cider made in 1904 three yeasts, *A*, *B* and *C*, were obtained:—

Yeast A. *Morphology.* Yeast *A* appeared on plate cultures as spherical dry colonies, with slightly crenate edges; it has a characteristic streak, heaped up on the gelatine, with sharply sloping corrugated sides, and a tendency to fringe on the edges, the whole growth having a creamy, solid-looking appearance. (Pl. VII, Fig. 70.) Liquefaction of the gelatine sometimes takes place after some considerable period.

Vegetation. Young cultures in beer-wort show fairly uniform oval cells. Size $8{\cdot}5 \times 6{\cdot}1\ \mu$. (Pl. I, Fig. 1.)

Young streak cultures consist of similar cells, but more sausage-shaped cells occur and the contents are distinctly more granular.

Old streak cultures. Cells of very various shapes and sizes occur and the contents are either coarsely granular, or sometimes collected into large globules. A good many spores are to be found, which are spherical bodies occurring 2, 3 or 4 in a mother-cell. (Pl. I, Fig. 2.)

Sedimentary. Cells oval and elongated in shape with more or less granular or vacuolate contents. (Pl. I, Fig. 3.)

Spore formation. This species forms spores fairly readily on potato and abundantly on porous porcelain at the ordinary temperature of the laboratory after about 90 hours.

Spores are also formed at lower temperatures, *e.g.* 15° C., but none are found in cultures kept at 26° C. (Pl. I, Fig. 4.) Size $3{\cdot}1\ \mu$.

Germination of spores. Pl. I, Fig. 5 shows stages in germination.

The mother-cell bursts and the swollen spores in many cases begin budding while still surrounded by the remains of the old wall.

Budding takes place in a very simple manner, each cell giving off a terminal bud which enlarges, and then a lateral bud is given off close to the old one. (Pl. I, Fig. 6.)

Physiology. Max. temp. between 35° and 38° C., only a moderate growth taking place at the former temperature and none at all at the latter.

Fermentations. Ferments dextrose, saccharose, laevulose and maltose.

Yeast B. *Morphology.* Yeast *B* resembles *A* very closely in most of its characters. The colonies on plate cultures are spherical, dry and tend to fringe at the edges. The streak (Pl. VII, Fig. 71) is indistinguishable from that of *A* at some stages, although it appears to grow

rather more slowly, and invariably liquefies gelatine after a time, a property which is not so constant in *A*, in which it appears to depend largely upon the freshness of the gelatine medium.

Vegetation. Young cultures in beer-wort consist of cells similar to those of *A* in appearance, but they have rather different dimensions— $6{\cdot}8 \times 10{\cdot}2 - 4{\cdot}4\ \mu$. (Pl. I, Fig. 7.)

Young streak cultures. Vegetation is identical with that of *A* at the same age.

Old streak cultures which have become liquid contain numerous spores occurring usually 3 or 4 in a cell, but in some elongated cells as many as 7, 8, or even 14 may be found, this being another point which separates this species from yeast *A*. (Pl. I, Fig. 8.)

The ordinary vegetative cells are vacuolate or coarsely granular in appearance, but the large globules which occur in *A* are almost entirely absent.

Sedimentary. Cells spherical and oval in shape with vacuolate, granular or homogeneous contents, *i.e.* the type of vegetation is very similar to that of *A*, except that no elongated cells occur. (Pl. II, Fig. 9.)

Spore formation can be induced fairly easily on carrot and also on porous porcelain, by the latter method spores being obtained after 42 hours at 26° C. and after 90 hours at the ordinary temperature; a few are also formed at 14·7° C. Pl. II, Fig. 10 shows the spores as they occur on carrot, and it will be seen that in some cases the cells containing them have a curious shape, and apparently all the protoplasm of the mother-cell is not used up in their formation, as a distinct granular mass is present in the swollen end of the cell. Size $3{\cdot}9\ \mu$.

Germination of spores. Stages such as those figured in Pl. I, Fig. 11 are often found in which the spores are escaping from the ruptured mother-cell.

Budding. The method is indistinguishable from that which occurs in *A*. (Pl. I, Fig. 12.)

Physiology. Max. temp. about 35° C.

Fermentations. Ferments dextrose, laevulose, saccharose and maltose.

Yeast C. *Morphology.* This is quite a distinct variety from *A* and *B*, forming spherical, moist and slightly transparent colonies on plate cultures, and a characteristic moist spreading streak with corrugated and scalloped edges. (Pl. VII, Fig. 72.) During the early stages of its

growth the surface of the streak was perfectly smooth, but in older cultures it has entirely altered its appearance, and has become covered with a kind of bead-like eruption. This possibly may be due to prolonged cultivation on artificial media, as plate cultures made from the streak showed no indication of impurity.

Vegetation. In young beer-wort cultures small and medium oval cells predominate with an average size of $4{\cdot}5 \times 3{\cdot}7\ \mu$. (Pl. II, Fig. 13.)

Young gelatine cultures show very similar vegetation, except that small granules are often present either inside the vacuole or surrounding it.

In *old streaks* the cells are usually spherical or oval with scanty contents, often condensed into a granular mass at two opposite ends of the cell. (Pl. II, Fig. 14.)

Sedimentary vegetation consists of small, spherical and oval cells with homogeneous or slightly vacuolate contents, the spherical ones having 3 or 4 buds attached. (Pl. II, Fig. 15.)

Spore formation. No spores have been observed in any cultures of this yeast; it must therefore be classed as a Torula form.

Budding. The type of budding is very characteristic, but the process is very difficult to follow under the microscope as the cells are so small and the buds slide over one another owing to the slight liquefaction of the gelatine in the immediate vicinity of the cells. (Pl. II, Fig. 16.)

Physiology. Max. temp. between 35° and 37·5° C.

Fermentations. Ferments dextrose, laevulose, saccharose and maltose.

From Sweet Alford Cider made in 1905 three yeasts, *D*, *E* and *F*, were isolated:—

Yeast D. *Morphology.* Yeast *D* may be distinguished on account of its dry spherical frosted colonies, which later show a tendency to flatten out on the gelatine, the edges becoming fringed and irregular. The streak shows a similar frosted appearance at first but later becomes rather moist and almost flat with slightly fringed edges. (Pl. VII, Fig. 73.)

Vegetation. Young beer-wort cultures. Oval cells of average size $4{\cdot}5 \times 3{\cdot}7\ \mu$ are most common, although a few elongated rod-like cells occur. (Pl. II, Fig. 17.)

Later this yeast forms a film on the surface of the beer-wort, the cells being oval and usually with a large central vacuole and a few refringent granules. (Pl. II, Fig. 20.)

Young streak cultures show uniformly oval and sausage-shaped cells, many with conspicuous vacuoles and bright granules.

In *old streaks* the vegetation is not very different, the cells retaining most of their original characters; in fact this species seems to be remarkable for the extreme uniformity of the vegetation on different media and at different ages. (Pl. II, Fig. 18.)

Sedimentary. Cells medium size, oval, with more or less vacuolate and granular contents; a few elongated ones occur which remain attached to one another in chains. (Pl. II, Fig. 19.)

Spore formation. No spores have been observed on any of the media used.

Budding. The method of budding is fairly simple; terminal buds are given off from two cells placed end to end, and then the process continues in both directions, terminal and lateral buds being formed. (Pl. II, Fig. 21.)

Physiology. Max. temp. at 32° C., at which temperature practically no growth takes place.

Fermentation. This yeast forms a film on all the sugars but does not induce any fermentation.

Yeast E. *Morphology.* Yeast *E* appears on plate cultures as spherical moist colonies with a tendency to be transparent. Streak cultures are almost indistinguishable from yeast *D*. (Pl. VII, Fig. 74.)

Vegetation. Young beer-wort cultures show oval cells of medium size ($11{\cdot}9 - 6{\cdot}8 \times 3{\cdot}4\,\mu$) with one or two vacuoles containing a few granules (Pl. II, Fig. 22), while in young streaks the cells are more homogeneous in appearance and no granules are present.

In *old streaks* the ordinary cells are medium size and oval, but about 50% are elongated, often with a bud at both ends, or connected together in chains (Pl. II, Fig. 23). The protoplasm is vacuolate, generally with a few refringent granules.

Sedimentary vegetation is very slight and consists of small and medium size oval and sausage-shaped cells with vacuolate contents and conspicuous food bodies. (Pl. II, Fig. 24.) This form, like *D*, forms a film on beer-wort, most of the cells being oval and homogeneous but with one or two large and conspicuous granules. (Pl. III, Fig. 25.)

Spore formation. This species, like *C* and *D*, is a Torula yeast, no spores being formed.

Budding. The type of budding is identical with that described for yeast *D*. (Pl. III, Fig. 26.)

Physiology. Max. temp. apparently about 38° C., so that it is a distinctly high temperature form.

Fermentation. No fermentation takes place with any of the sugars used, but a well-marked film is formed on the maltose solution.

Yeast F. *Morphology.* Yeast *F* forms spherical solid-looking colonies with smooth edges, and a slightly moist, milky-looking streak with smooth surface and edges and a fairly rapid rate of growth. (Pl. VII, Fig. 75.) This proved to be an exceedingly interesting type, in that it was found to be conjugating in plate cultures only a few days old.

Vegetation. In *young beer-wort cultures* the cells are of the ordinary oval shape (size 6·8 × 3·4 μ) and are very homogeneous in appearance (Pl. III, Fig. 27), but in young gelatine cultures the contents are much more vacuolate and usually several conspicuous granules are present.

In *old streaks* the cells are usually of medium size, many of them spherical, with several buds attached, and all stages in conjugation and subsequent spore formation can be found. (Pl. III, Figs. 28 and 30.)

Conjugation seems more or less a response to the stimulus of starvation, because it takes place after a much longer time on streaks than on plate cultures; and the time required is certainly longer now, after long continued cultivation on artificial media, than on the first isolation of the yeast, possibly because it has become accustomed to its present conditions of culture. The process of conjugation has not been followed under the microscope, but observation of the various stages found makes it practically certain that beaks put out from two cells meet and fuse, and then the fused contents proceed to form spores, as in Zygosaccharomyces. (Barker, 2.) Normally two spores are formed in each compartment of the compound cell, but occasionally three are formed in one, and only one in the other, the distribution probably being connected with the relative sizes of the conjugating cells. Occasionally the fused cells have buds attached to them, which probably had been formed prior to conjugation.

Sedimentary vegetation consists of medium sized oval and small sausage-shaped cells with rather variable contents, homogeneous, vacuolate and granular types of cells being found. (Pl. III, Fig. 29.)

Spore formation. Conjugation and spore formation takes place on porous blocks and in cultures on potato, as well as in streak cultures on beer-wort gelatine. Size of spores 3·1 μ.

Germination of spores. On germination the spores swell up, and the wall of the mother-cell bursts in both compartments and the spores begin to bud while still partly enclosed. (Pl. III, Fig. 31.)

Budding. The method of budding is not very distinctive. A terminal bud is formed and grows to the same size as the parent cell and then each cell goes on budding independently, although as a rule only one bud is formed from a given cell. (Pl. III, Fig. 32.)

Physiology. Max. temp. between 30° and 32·5° C.

Fermentations. Ferments dextrose, laevulose and saccharose.

From Sweet Alford Cider made in 1906 four yeasts, *G, H, I* and *J*, were isolated:—

Yeast G. *Morphology.* Yeast *G* occurs as dry spherical colonies with a wrinkled surface, and the streak has a dry, yellowish appearance, slightly heaped in the middle and spreading out towards the edges, the latter being slightly crenate. (Pl. VII, Fig. 76.)

Vegetation. In *young beer-wort* the vegetation consists of vacuolate, oval cells with one or two conspicuous granules and an average size of $7{\cdot}8 - 5 \times 5 - 4{\cdot}1\ \mu$. (Pl. III, Fig. 33.)

In *young streaks* sausage-shaped cells are more numerous.

In *old streaks* the cells vary from spherical to sausage-shaped, with rather variable contents. (Pl. III, Fig. 34.)

This yeast gives rise to a film upon the surface of beer-wort after a short time, but the cells are very similar to those in young beer-wort cultures except that the central vacuole is somewhat more definite. (Pl. III, Fig. 36.)

The *sedimentary* growth does not show any essential differences from that of young beer-wort cultures. (Pl. III, Fig. 35.)

Spore formation. In some old streak cultures suggestions of conjugation were observed, and on cultivating the yeast on porous porcelain in the absence of CO_2 this was confirmed, and the cells were obtained in various stages of conjugation. Stages in this process are shown in Pl. III, Fig. 37, and it will be noticed that the spores are only formed in *one* compartment of the compound cell. This seems to be the rule for this species, as only in very rare cases were spores observed in both of the fusing cells. If this proves to be usual, it is interesting, inasmuch as it rather indicates a certain physiological differentiation of sex between the cells. In some cases apparently only one spore is formed, although that appearance may be due to the plane of formation of the spores, the other one being hidden from view. The spores are fairly large, $4{\cdot}3\ \mu$ in diameter.

Germination of spores. Stages in germination of the spores are shown in Pl. IV, Fig. 38, the process taking place in quite the normal way.

Budding. The budding process is almost identical with that described for *A* and *B*. (Pl. IV, Fig. 39.)

Physiology. Max. temp. about 32·5° C.

Fermentation. No fermentation takes place in any of the sugars, but films are formed on the surfaces of the liquids.

Yeast II. *Morphology.* Yeast *H* is distinguished by the formation of spherical dry white colonies on plate cultures, and a somewhat moist-looking streak, with corrugated slopes and fringed edges. It has a fairly rapid rate of growth and causes liquefaction of the gelatine after a time. (Pl. VII, Fig. 77.)

Vegetation. The vegetation in *young cultures*, both in liquid and on solid media, is very similar, consisting of oval and rather pointed cells, either homogeneous or with small vacuoles, and also some enlarged oval cells with a large central vacuole. The average sizes are 10·8 – 9·5 – 5·1 × 6·8 × 5·1 × 3·7 μ. (Pl. IV, Fig. 40.)

Old beer-wort gelatine streak cultures show cells of various sizes and shapes (Pl. IV, Fig. 41); many are elongated, and a few hour-glass-shaped cells occur, suggestive of conjugation, although no spores have been observed in them, while ordinary endospore formation takes place fairly readily both on porcelain blocks at the ordinary temperature and on potato and carrot, as well as in old streaks. The spores occur 2, 3 or 4 in a cell, and have a diameter of 2·3 μ. (Pl. IV, Fig. 43.)

Sedimentary vegetation consists of medium to large oval and irregular sausage-shaped cells with vacuolate contents, or homogeneous with refringent granules.

Germination of spores takes place in the usual way, as seen in Pl. IV, Fig. 44, but in elongated cells there seems to be a kind of attachment between the spores, although it is probable that there is no actual fusion between them, such as takes place in *Saccharomyces Ludwigii*. (Guilliermond, 5.)

Budding. The method of budding is rather like that described for yeast *F*, but somewhat less regular, probably owing to slight liquefaction of the gelatine. (Pl. IV, Fig. 45.)

Physiology. Max. temp. between 30° and 32° C., as no growth takes place at the higher temperature.

Fermentations. Dextrose, laevulose, maltose and saccharose are attacked.

Yeast I. *Morphology.* Yeast *I* has cone-shaped colonies with ridges on the surface and rather fringed edges, and the streak has a

moist creamy appearance, with feathered edges, and about the same rate of growth as *H.* (Pl. VII, Fig. 78.)

Vegetation. Young beer-wort cultures are characterised by medium sized ovoid cells ($13{\cdot}6 - 6 \times 6 - 5\ \mu$), with vacuolate or slightly granular contents. (Pl. IV, Fig. 46.)

The vegetation of young streaks is similar, except that the cells are less uniform in shape.

Old streak cultures are characterised by the remarkable abundance of spores; in fact the main distinguishing feature of this species is its extreme readiness to form spores on all the media used. The ordinary cells are ovoid or sausage-shaped with faint granular contents. (Pl. IV, Fig. 47.)

Sedimentary vegetation consists of medium sized oval and sausage-shaped cells with vacuolate and granular contents. (Pl. IV, Fig. 48.)

Spore formation, as mentioned above, takes place very quickly, spores being found in colonies on plate cultures, and using the usual plaster block method a good number of cells were found containing spores after 22 hours at 26° C.

The spores are $3{\cdot}5\ \mu$ in diameter and are formed from two to four in the mother-cell, and many of them show a dark area in the middle. (Pl. IV, Fig. 49.)

Germination of spores. On germination the spores swell up until the wall of the mother-cell becomes stretched and thin; it then bursts and the spores are liberated. Apparently small buds are often formed before the escape of the spores, as a sort of cap may be seen on them sometimes while still within the mother-cell. (Pl. IV, Fig. 50.)

Budding. The method of budding does not show any very distinctive characters, except that the cells of the colony seem to hold together very loosely. (Pl. V, Fig. 51.)

Physiology. Max. temp. between 35° and 38° C.

Fermentations. Identical with those of yeast *H.*

Yeast J. *Morphology.* Yeast *J* is a rapidly growing species forming spherical, moist, semi-transparent colonies, a flat, spreading streak of similar appearance. (Pl. VII, Fig. 79.) Old streaks become covered with an eruption something like that observed on the streak of yeast *C*, but apparently in this case also, it is not due to the presence of an impurity.

Vegetation. The vegetation is characteristic:—

In *young beer-wort cultures* it consists of small oval or spherical cells

with a central vacuole and an average size of $8{\cdot}5 - 6{\cdot}8 \times 4 - 3{\cdot}5\ \mu$. (Pl. V, Fig. 52.) Much the same type of cell occurs on solid media except that the vacuole is usually absent.

Old streaks consist of small ovoid, spherical cells with vacuolate and granular contents (Pl. V, Fig. 53), and the *sedimentary vegetation* is essentially the same. (Pl. V, Fig. 54.)

Spore formation. No spores have been found in any of the cultures, so that this species forms another addition to the list of Torula varieties.

Budding. The method of budding is rather irregular, resulting in more or less spherical groups of cells. (Pl. V, Fig. 55.)

Physiology. Max. temp. between 30° and 32° C.

Fermentations. Dextrose, laevulose and saccharose only are fermented.

From Kingston Black Cider made in 1904 two yeasts, *K* and *L*, were isolated:—

Yeast K. *Morphology.* Yeast *K* appears as dry spherical colonies with smooth edges, almost indistinguishable from yeast *A*, but it was found that the two species could be separated on the characters of the streaks as that of *K* is very much more heaped up on the gelatine and has smooth, steep slopes and fairly regular edges. (Pl. VII, Fig. 80.)

Vegetation. In *young beer-wort cultures* the cells are oval and sausage-shaped, the representative sizes being $13{\cdot}9 \times 3{\cdot}7$ and $8{\cdot}5 \times 3{\cdot}7\ \mu$. (Pl. V, Fig. 56.) This form produces very active fermentation in the liquid.

In *young streaks* the vegetation is very similar but rather more granular.

In *old streaks* cells of very varied shapes occur (Pl. V, Fig. 57), and the protoplasmic contents generally show a central vacuole surrounded by a ring of granules; a very few spores also are found.

Sedimentary vegetation consists of medium to large oval spherical and sausage-shaped cells with vacuolate or very granular contents. (Pl. V, Fig. 58.)

Spore formation takes place in cultures on porous blocks at 26° C. after 43 hours and at the ordinary temperature after 90 hours. Spores are also formed on carrot and potato at 26° C.

The spores occur two to four in a cell, and have a diameter of $3{\cdot}9\ \mu$. (Pl. V, Fig. 59.)

Germination of spores. Germination takes place in the usual way. (Pl. V, Fig. 60.)

Budding. The type of budding is rather characteristic, several buds arising in succession (often in pairs) from the same cell, so that a compact spherical mass of cells results. (Pl. V, Fig. 61.)

Physiology. Max. temp. about 38° C., at which temperature a very slight growth takes place.

Fermentations. Ferments dextrose, laevulose, maltose and saccharose.

Yeast L. *Morphology.* Yeast *L* is distinguished from all the other species isolated by its extremely slow rate of growth. After several days minute spherical moist colonies with smooth edges appeared, which gave rise to a smooth compact streak. (Pl. VII, Fig. 81.)

Vegetation. The *young vegetation* is fairly uniform in both liquid and solid media, consisting of small to medium sized ($12 - 6{\cdot}8 \times 2{\cdot}7\ \mu$) slender sausage-shaped cells. (Pl. V, Fig. 62.)

In *old streaks* the vegetation still retains much the same characters. (Pl. VI, Fig. 63.)

Sedimentary vegetation. The cells have a very similar appearance (Pl. VI, Fig. 64) to that which they have in the other cultures described, so that this form, like yeast *D*, is fairly constant, and does not vary appreciably with age and the nature of the culture medium.

Spore formation. No spores have been observed in this species.

Budding. The method of budding resembles that in yeasts *E* and *H*. (Pl. VI, Fig. 65.)

Physiology. Max. temp. above 38° C., at which temperature a vigorous growth takes place; this species therefore is distinctly a high temperature form.

Fermentations. Dextrose and maltose are the only sugars fermented.

From Kingston Black Cider made in 1906 three yeasts, *M* and two others, were obtained:—

Yeast M. *Morphology.* Yeast *M* occurs as dry spherical, solid-looking colonies with smooth edges, and possesses a moist, slightly heaped streak with slightly corrugated slopes and fringed margin. The gelatine, however, becomes liquid after a time.

Vegetation. The vegetation in *young beer-wort* and *young gelatine cultures* consists of medium sized ($8 - 6{\cdot}8 \times 5\ \mu$) oval cells, generally with a central vacuole containing a few granules. (Pl. VI, Fig. 66.)

In *old streaks* the cells are oval or sausage-shaped with granular contents. (Pl. VI, Fig. 67.)

A	Streak compact, opaque and raised: surface corrugated and dry: edges somewhat fringed	Fairly uniform ovoid: about $8{\cdot}5 \times 6{\cdot}1\ \mu$	Various: many cells containing spores: $21 - 7 \times 4 - 7\ \mu$	Abundant	—	32·5—35° C.	Dextrose, laevulose, saccharose, maltose	Fairly compact but irregular
B	As *A*, but grows rather more slowly and liquefies gelatine more readily	As *A*: $10{\cdot}5 - 6{\cdot}5 \times 4 - 7\ \mu$	Various: spores abundant, some cells containing as many as 14	Abundant	—	About 32·5° C.	As *A*	Irregular, loos branching color rather spreadin
C	Streak spreading, semi-transparent and flat: surface originally smooth and moist: edges irregular and fringed	Small and medium ovoid or slightly spherical: about $4{\cdot}5 \times 3{\cdot}7\ \mu$	Spherical and ovoid	None observed	—	35—37° C.	As *A*	Small, spheric and compact
D	Streak opaque, frosted and slightly raised, when young, becoming flattened later: surface at first dry and slightly rough, later somewhat moist and smooth: edges irregular and fringed	Mainly small ovoid, with some sausage-shaped and more elongated: about $4{\cdot}5 \times 3{\cdot}7\ \mu$	As in young cultures, but cells more granular and nearly empty	None observed	In young cultures on all liquid nutrient media	31—32·5° C.	None	Irregular and slightly sprea ing
E	Streak almost indistinguishable from *D*	Fairly uniform ovoid: about $6{\cdot}8 - 11{\cdot}9 \times 3{\cdot}4\ \mu$	Ovoid and elongated cells in about equal numbers: protoplasm vacuolate with refringent granules	None observed	On beer-wort and maltose solution	About 38° C.	None	Similar to *D* b rather more compact
F	Streak slightly moist, milky-looking, semi-transparent, flat and spreading: surface and edges smooth	Uniform ovoid: about $6{\cdot}8 \times 3{\cdot}4\ \mu$	Ovoid and spherical cells, the latter often with several buds attached. Conjugation	Moderate, after some time. Conjugation	—	30—32·5° C.	Dextrose, laevulose, saccharose	Cells arranged loosely: colo irregular
G	Streak opaque, dry, yellowish, slightly raised in middle and spreading out towards edges: edges very slightly crenate	Ovoid, with one or two conspicuous granules: about $7{\cdot}8 - 5 \times 5 - 4{\cdot}1\ \mu$	Spherical to sausage-shaped: conjugation occasionally seen	Abundant spores in one culture, succeeding conjugation	On beer-wort and other nutrient solutions	About 32·5° C.	None	Fairly compact, but irregular shape
H	Streak rather moist, fairly compact, somewhat raised, with sloping sides corrugated and edges fringed	Ovoid and rather pointed cells: $10{\cdot}8 - 5{\cdot}1 \times 6{\cdot}8 - 3{\cdot}7\ \mu$	Various, especially ovoid, elongated and hour-glass-shaped: spores fairly abundant	Fairly abundant	—	30—32° C.	As *A*	Compact, causi slight liquefi tion of gelati
I	Streak moist and creamy; compact, slightly raised, with corrugated slopes and feathered edges	Ovoid and fairly uniform: $13{\cdot}6 - 6{\cdot}1 \times 6{\cdot}1 - 5{\cdot}1\ \mu$	Ovoid and sausage-shaped, with abundant spores	Very abundant	—	35—38° C.	As *A*	Elongated and spreading
J	Streak moist and spreading: in early stages surface smooth, but later covered with bead-like eruptions: edges smooth	Ovoid and spherical: $8{\cdot}5 - 6{\cdot}8 \times 4 - 3{\cdot}7\ \mu$	Ovoid and spherical with vacuolate and granular contents	None observed	—	30—32° C.	As *F*	Compact and spherical
K	Streak dry, solid-looking, very compact and raised, with fairly smooth sloping sides and regular edges	Ovoid and sausage-shaped: about $13{\cdot}9 - 8{\cdot}5 \times 3{\cdot}7\ \mu$	Various, with central vacuole and ring of granules: spores few	Few	—	About 38° C.	As *A*	Very compact a spherical
L	Streak moist, compact and raised, with surface and edges smooth	Uniform slender sausage-shaped: about $11{\cdot}9 - 6{\cdot}8 \times 2{\cdot}7\ \mu$	Very similar to young cultures in shape	None observed	—	Above 38° C.	Dextrose, maltose	Rather elongat but fairly comp
M	Streak moist, compact and slightly [illegible]	Uniform ovoid: about	Ovoid and sausage-	None observed		35—38° C.	As *A*	Compact, causi liquefaction of

Sedimentary vegetation shows medium sized oval cells, most of which have extremely granular contents, although some are vacuolate. (Pl. VI, Fig. 68.)

Spore formation. No spores have been observed in any of the cultures.

Budding. The method of budding is difficult to follow, because in this yeast, as in yeast *C* (and for the same reason), the buds are formed on top of one another. The best series of stages obtained are given in Pl. VI, Fig. 69.

Physiology. Max. temp. between 35° and 38° C.

Fermentations. Ferments dextrose, laevulose, maltose and saccharose.

The two other species isolated from this cider proved on analysis to be identical with *A* and *C* respectively.

General considerations.

Reviewing the results above described it will be seen that three varieties of yeast were isolated from the 1904 Sweet Alford Cider, three from the 1905 Sweet Alford, four from the 1906 Sweet Alford, two from the 1904 Kingston Black and three from the 1906 Kingston Black, making a total of 15 in all. Of these 11 are undoubtedly distinct varieties, while two kinds found in the 1906 Kingston Black are apparently identical with two—*A* and *C*—isolated from the 1904 Sweet Alford. There were thus at least 13 different yeasts in the five samples, and only in the instances just mentioned were the same yeasts found in different ciders.

It is of course possible that in each cider examined yeasts other than those isolated were present; but it is extremely improbable that any such forms occurred in appreciable numbers, or constituted more than a very limited percentage of the total yeast vegetation, since the original series of plate cultures were examined very carefully and the types isolated were apparently the only forms occurring on the plates. It may at any rate fairly be claimed that the varieties isolated in each instance constituted practically the whole of the active vegetation in the ciders at the time of examination. With the history of the vegetation of these ciders prior to that time the present investigation was not concerned, but it may be surmised that at any rate the larger, actively fermenting forms, such as *A*, *B* and *H*, played a prominent, if not predominant, part in the earlier stages of fermentation. The smaller, non-

fermenting or feebly-fermenting types, such as *D*, may not have exerted so potent an influence on the character of the primary fermentation, whatever they might do in the later stages; and it is possible that certain types which take an active part in the early stages may have become practically extinct in the mature cider. For instance, it is undoubtedly the case that varieties of *Saccharomyces apiculatus* are fairly regularly to be found in ciders only two or three months old; but in no case was a yeast of the *apiculatus* type found in the ciders examined, thus leading to the presumption that such forms gradually die out or become dormant, as the liquor matures, a view supported by the observations of Kayser (6) and Dienert (4). It may be doubted, however, if any forms which play a predominant part in the early stages of fermentation become sufficiently reduced in numbers in the later stages to be overlooked entirely.

Taking, however, the results as they stand, they afford no support to the ideas that certain yeasts may be regularly associated with certain apples or that the fermentations in a cider factory may be carried on mainly by any group of yeasts which may have a regular habitat there.

Regarding the former point the Sweet Alford apple is extensively grown in Devon, so that, although the samples of fruit from which the ciders in question were made came from different districts in that county, it might be expected that the supposed organisms common to the variety would be present on the fruit in each instance, if the supposition of their existence is justified. Their presence would be additionally probable, since the samples did not consist of a few specimens of fruit only, but were comprised in each case of about half a ton of the fruit, the produce, probably, not of a single tree alone, but of many. And similarly with the Kingston Black apple. This variety is more widely grown than any other cider apple, and occurs probably in every cider-growing centre of any importance in the West of England. There are, therefore, two alternatives which arise. Either this supposed association of particular yeasts with special kinds of apples does not exist, or these common organisms have become obsolete in the ciders examined before the time of the investigation. While it would be going too far to declare absolutely in favour of the former alternative, it cannot be denied that the results point very strongly in that direction. Granting for the sake of argument the association of special yeasts with particular varieties of apples, it possesses no significance of any practical importance unless those yeasts exercise a controlling influence upon the character of the fermentation. If they are sufficiently numerous and

powerful to do that, then their presence in the later stages of fermentation would be extremely likely, more especially since it is during that period that the yeast apparently exerts most powerfully its characteristic influence upon the development of aroma and flavour. It was during that period that the samples selected were examined.

But, since the results show that no common forms occur either in the three Sweet Alford or the two Kingston Black Ciders respectively, the possibility of their occurrence to a significant extent at any stage of fermentation is greatly weakened: and, even admitting their presence in the earlier stages, it is difficult to see how they could impart any special character to the nature of the fermentation. Thus it appears as if the question of the occurrence of such associations is not of importance in practical cider making; and that, further, no evidence has been found to indicate their existence.

The question of the possible influence of yeasts having their habitat in the cider factory stands in some respects in the same position as the foregoing. If any single one or group of such forms played a dominant part in the fermentation, evidence of its or their existence in the bottled samples would have been expected. The only yeasts which were found in more than one sample are *A* and *C*. These were present in the 1904 Sweet Alford and the 1906 Kingston Black Ciders. Since a two-years interval elapsed between the making of these ciders, the presence of two similar yeasts in both cannot *per se* be considered to indicate that those forms dominate the fermentations at the Institute regularly; and moreover there is no evidence of their presence in the other ciders examined, either in those made in the same seasons, 1904 and 1906, or in the intermediate season of 1905. A more likely explanation of their occurrence is that possibly the same cask might have been used for both ciders concerned at some stage of their manufacture. It is difficult to sterilise casks thoroughly and, although all used in the making of these ciders were carefully washed and steamed before use, doubtless many germs remaining from previous contents survived the treatment and developed in the ciders placed in those casks subsequently. If this is the case, the same yeasts would occur to some extent in the various ciders for which a cask was used, and the presence of the two forms in the 1904 Sweet Alford and the 1906 Kingston Black Ciders might perhaps be accounted for thus, although nothing definite can be said, since unfortunately it could not be traced if the same cask was at any time used for the two ciders.

The results also give no support to the idea that certain yeasts may

be especially prevalent in one season and may dominate the fermentations in that season, while others may fill their place in other seasons. There is no indication of a common form having at any time been present in the 1904 or the 1906 Sweet Alford and Kingston Black Ciders. It is of course possible that some yeasts do occur more abundantly in some seasons than in others: but, if that is so, it still remains to be proved that the point has any practical bearing on cider fermentations.

The chemical characters of the unfermented juices from which the ciders were made, as well as of the mature ciders themselves, may influence the nature of the yeast flora. The only obvious marked difference in composition between the Sweet Alford and Kingston Black Ciders used in this investigation was in the acidity. The fresh unfermented juices from the Sweet Alford samples contained an equivalent of ·15 – ·2 per cent. malic acid, while the Kingston Black juices showed percentages of ·5 – ·7 malic acid. Since, however, the yeasts concerned have on trial shown themselves to be able to grow apparently equally freely in liquids of both degrees of acidity, there seems to be no reason, in this case at least, for attaching much importance to the chemical composition of the juice as a selective factor. It is intended to deal with this question as well as that of the chemical and fermentative qualities of the individual yeasts in a future paper.

Since the various lots of fruit came from different localities, nothing definite can be said as to whether the yeast flora of the ciders was in any way representative of the yeast flora of the district in which the fruit was grown. It may be that such was the case, in which event there would appear to be a more or less definite factor to reckon with in cider fermentation work.

Failing that, there seems to be no other obvious source to which to look for an explanation of the character of the yeast flora of ciders. The five samples examined have floras so diverse, that it is clear that the fermentation of cider in the ordinary manner is most uncertain in character. One might perhaps be justified in suggesting that every separate cask of cider had a flora of its own. It is evident also that uniformity in the character of the product cannot be looked for under the old system and that at times the quality is certain to fall short of that which might have been obtained, if a selected yeast had been used to dominate the fermentation.

BIBLIOGRAPHY.

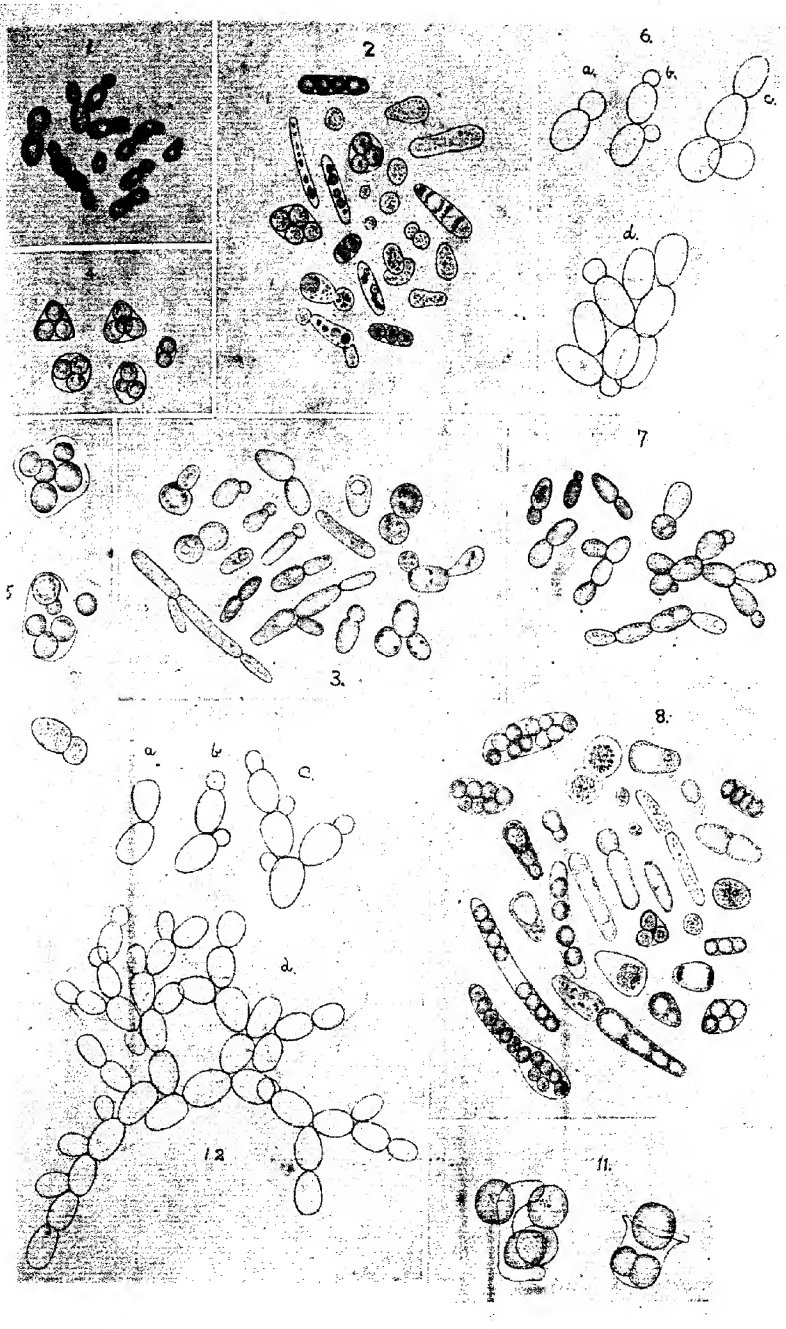
1. ALLWOOD, DAVIDSON and MONCURE. The Composition of Cider as determined by Dominant Fermentation with Pure Yeasts. *U.S. Dept. of Agriculture.* Bureau of Chemistry, Bulletin 88, 1904.
2. BARKER. A Conjugating Yeast. *Phil. Trans. Royal Soc.*, Ser. B, 203.
3. —— Annual Reports on Experimental Cider Work. *The National Fruit and Cider Institute*, 1905–1907.
4. DIENERT. *Le Cidre et le Poiré*, 1896.
5. GUILLIERMOND. *Recherches cytologiques sur les Moisissures*, Lyon, 1902.
6. KAYSER. Levures de Cidre. *Ann. Inst. Pasteur*, 1890.
7. JACQUEMIN and ALLIOT. La Cidrerie Moderne. Malzéville-Nancy, 1902.
8. JORGENSEN. *Microorganisms and Fermentation*, 3rd edit., London, 1900.
9. LLOYD. Report on Results of Investigations into Cider-making. *Board of Agriculture and Fisheries*, 1903.
10. WORTMANN. *Anwendung und Wirkung reiner Hefen in der Weinbereitung*, 1895.

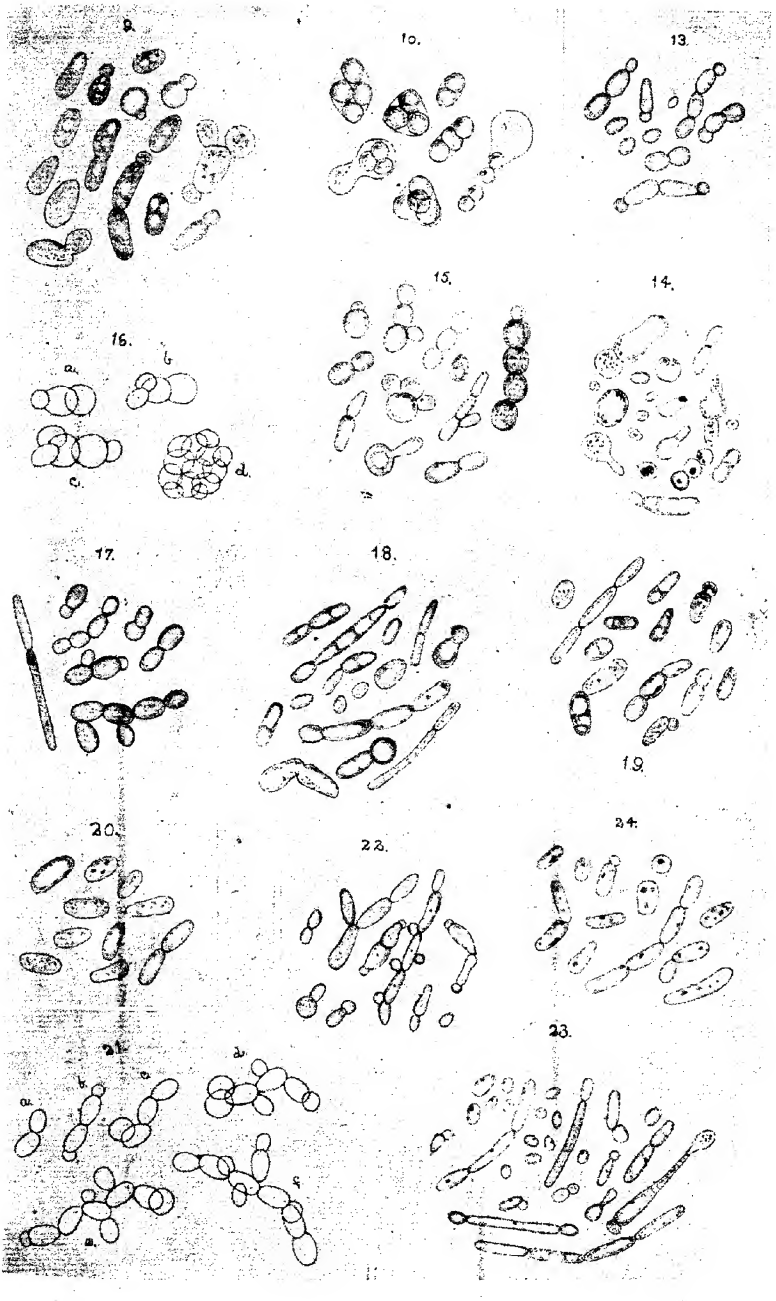
DESCRIPTION OF FIGURES.

Age of young beer-wort cultures = 24 hours.
,, old streak ,, = 4 months.
,, sedimentary ,, = 15 days.
,, films = 9 days.

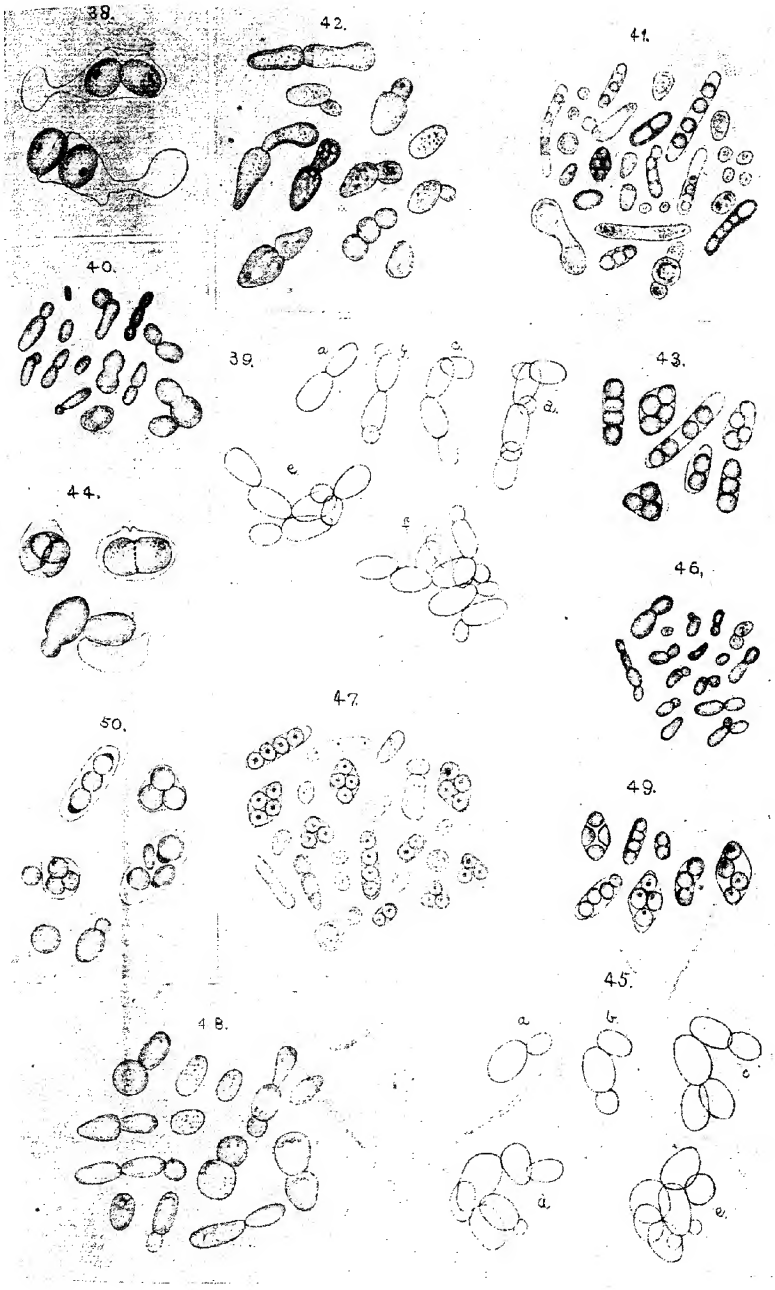
Yeast A.	Plate I.	Fig. 1.	Vegetation of young beer-wort culture. × 600.
	,,	,, 2.	,, old streak culture. × 750.
	,,	,, 3.	Sedimentary vegetation. × 750.
	,,	,, 4.	Spores from culture on potato. × 750.
	,,	,, 5.	Stages in germination of spores. × 1250.
	,,	,, 6.	*a, b, c, d.* Method of budding in hanging-drop culture. × 1250.
Yeast B.	,,	,, 7.	Vegetation of young beer-wort culture. × 750.
	,,	,, 8.	,, old streak culture. × 750.
	Plate II.	,, 9.	Sedimentary vegetation. × 750.
	,,	,, 10.	Spores from culture on carrot and from an old streak. × 750.
	Plate I.	,, 11.	Stages in germination of spores. × 1250.
	,,	,, 12.	*a, b, c, d.* Method of budding. × 1250.
Yeast C.	Plate II.	,, 13.	Vegetation of young beer-wort culture. × 850.
	,,	,, 14.	,, old streak culture. × 1000.
	,,	,, 15.	Sedimentary vegetation. × 1000.
	,,	,, 16.	*a, b, c, d.* Stages in budding. × 1250.
Yeast D.	,,	,, 17.	Vegetation of young beer-wort culture. × 850.
	,,	,, 18.	,, old streak culture. × 850.
	,,	,, 19.	Sedimentary vegetation. × 850.
	,,	,, 20.	Film vegetation. × 1000.
	,,	,, 21.	*a, b, c, d, e, f.* Stages in budding. × 1000.

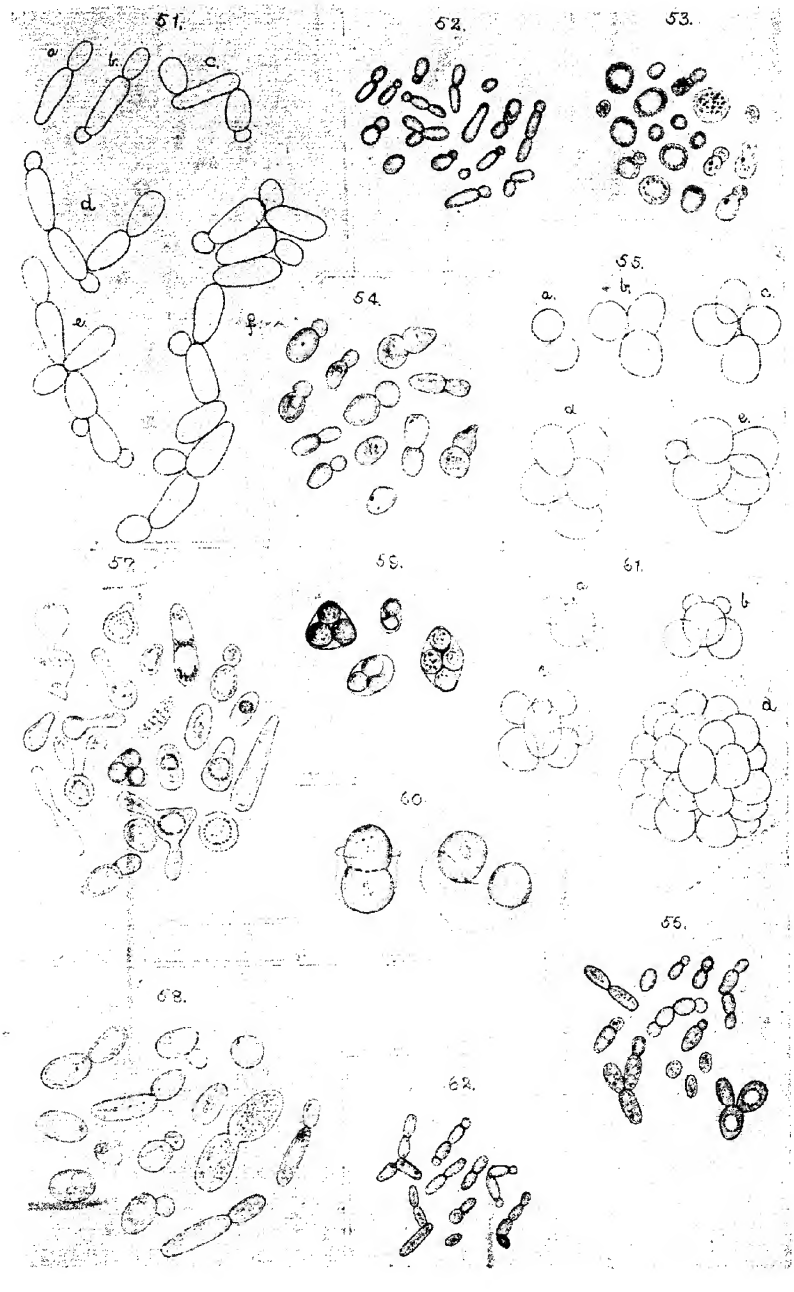
Yeast E.	Plate II.	Fig.	22.	Vegetation of young beer-wort culture. ×850.
	,,	,,	23.	,, old streak culture. ×850.
	,,	,,	24.	Sedimentary vegetation. ×850.
	Plate III.	,,	25.	Film vegetation. ×1000.
	,,	,,	26.	*a, b, c, d, e.* Stages in budding. ×1250.
Yeast F.	,,	,,	27.	Vegetation of young beer-wort culture. ×850.
	,,	,,	28.	,, old streak culture. ×1000.
	,,	,,	29.	Sedimentary vegetation. ×1000.
	,,	,,	30.	Stages in conjugation and spore formation (from old streak). ×1000.
	,,	,,	31.	Germination of spores. ×1250.
	,,	,,	32.	*a, b, c, d.* Stages in budding. ×1250.
Yeast G.	,,	,,	33.	Vegetation of young beer-wort culture. ×750.
	,,	,,	34.	,, old streak culture. ×1000.
	,,	,,	35.	Sedimentary vegetation. ×1000.
	,,	,,	36.	Film vegetation. ×1000.
	,,	,,	37.	Stages in conjugation and spore formation (from culture on porous porcelain). ×1000.
	Plate IV.	,,	38.	Germination of spores. ×1250.
	,,	,,	39.	*a, b, c, d, e, f.* Stages in budding. ×1250.
Yeast H.	,,	,,	40.	Vegetation of young beer-wort culture. ×600.
	,,	,,	41.	,, old streak culture. ×600.
	,,	,,	42.	Sedimentary vegetation. ×750.
	,,	,,	43.	Spores from culture on carrot. ×750.
	,,	,,	44.	Germination of spores. ×1250.
	,,	,,	45.	*a, b, c, d, e.* Stages in budding. ×1250.
Yeast I.	,,	,,	46.	Vegetation of young beer-wort culture. ×450.
	,,	,,	47.	,, old streak culture. ×600.
	,,	,,	48.	Sedimentary vegetation. ×750.
	,,	,,	49.	Spores from an old streak culture. ×750.
	,,	,,	50.	Germination of spores. ×850.
	Plate V.	,,	51.	*a, b, c, d, e, f.* Stages in budding. ×1250.
Yeast J.	,,	,,	52.	Vegetation of young beer-wort culture. ×450.
	,,	,,	53.	,, old streak culture. ×600.
	,,	,,	54.	Sedimentary vegetation. ×750.
	,,	,,	55.	*a, b, c, d, e.* Stages in budding. ×1250.
Yeast K.	,,	,,	56.	Vegetation of young beer-wort culture. ×600.
	,,	,,	57.	,, old streak culture. ×750.
	,,	,,	58.	Sedimentary vegetation. ×850.
	,,	,,	59.	Spores from culture on carrot. ×750.
	,,	,,	60.	Germination of spores. ×1250.
	,,	,,	61.	*a, b, c, d.* Stages in budding. ×1250.
Yeast L.	,,	,,	62.	Vegetation of young beer-wort culture. ×750.
	Plate VI.	,,	63.	,, old streak culture. ×1000.
	,,	,,	64.	Sedimentary vegetation. ×1250.
	,,	,,	65.	*a, b, c, d.* Stages in budding. ×1500.
Yeast M.	,,	,,	66.	Vegetation of young beer-wort culture. ×600.
	,,	,,	67.	,, old streak culture. ×750.
	,,	,,	68.	Sedimentary vegetation. ×850.
	,,	,,	69.	*a, b, c, d, e.* Stages in budding. ×1250.


1
2
6
a.
b.
c.
d.
7
3.
8.
a.
b.
c.
d.
12
11.


8.
10.
13.
15.
14.
16.
a.
b.
c.
d.
17.
18.
19.
20.
22.
24.
21.
a.
b.
c.
d.
e.
f.
g.
23.


25.
a
b
c
d
e
26.
29.
27.
32.
a
b
c
30.
d
33.
35.
36.
34.
31.
37.
28.


38.
42.
41.
40.
39.
a
b
c
d
e
f
43.
44.
46.
47.
50.
49.
48.
45.
a
b
c
d
e


51.
a
b
c
d
e
52.
53.
54.
55.
a
b
c
d
e
56.
57.
58.
59.
60.
61.
a
b
c
62.
63.
64.
65.


64.
68.
67.
63.
65.
a
b
c
d
66.
69.
a
b
c
d
e

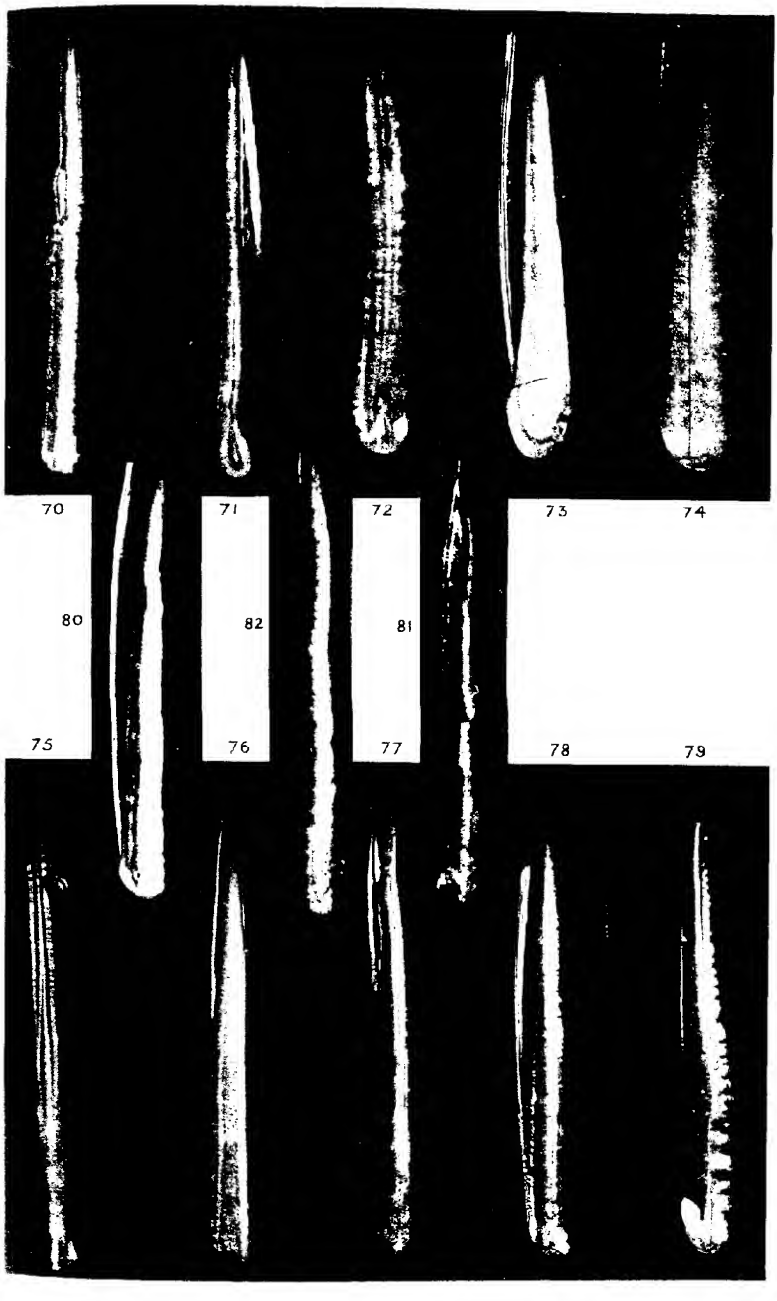

70
71
72
73
74
80
82
81
75
76
77
78
79

Plate VII. Photographs of streak cultures.

A = Fig. 70, natural size.	*H* = Fig. 77, natural size.
B = ,, 71 ,,	*I* = ,, 78 ,,
C = ,, 72 ,,	*J* = ,, 79 ,,
D = ,, 73 ,,	*K* = ,, 80 ,,
E = ,, 74 ,,	*L* = ,, 81 ,,
F = ,, 75 ,,	*M* = ,, 82 ,,
G = ,, 76 ,,	

A and *E*, nearly 5 weeks old.
B, *H* and *M*, 3 weeks old.
C, *D*, *F*, *G*, *I*, *J*, *K* and *L*, 5½ weeks old

THE GENETIC CLASSIFICATION OF SOILS.

By N. M. TULAÏKOFF (*Agric. Inst., Moscow, Russia*).

All classifications of soils yet proposed may be divided into two groups: (*a*) *scientific classifications* which are based on the natural characteristic of the soil, and (*b*) "*applied*" *classifications* which are based on the suitability of soils for certain crops, or on the revenue that may be derived from them.

According to the features on which the study of soils is based, the scientific classifications are divided into

(1) *the geologico-petrographical*, in which the soils are grouped according to the geologico-petrographical character of the rocks which make up the soil. (The classification of Fallou, Mayer and others.)

(2) *the chemical* or *chemico-petro-graphical*, according to the main chemical features of the soil (Knop).

(3) *the physical*, according to the mechanical composition and the physical characteristics derived from it. (Thaer, Schübler, the classification adopted by the Bureau of Soils for the investigation of soils in the United States of America.)

(4) *the combined classifications*, by which soils are divided into groups, for example according to their mechanical composition, and subdivided according to either their chemical composition or other features. (Senft, Kostichcff, and others.)

(5) *the genetic*, by which soils are divided into groups depending on their origin and development. (Prof. Docuchaiev, Prof. Hilgard, Prof. Ramman (in part), and Prof. Sibirtzev.)

In the present article I cannot touch on the applied classifications, or on the first four groups of the scientific classification; I shall, therefore, confine myself solely to the last, i.e. the genetic classification of Prof. Sibirtzev, which is the classification of Prof. Docuchaiev enlarged and improved. Prof. Hilgard's work in the classification of

soils is confined to the fundamental groups (Sedentary, Colluvial and Alluvial soils).

The scientific, but not the applied definition of soil, must emphasize its characteristic peculiarities as a natural substance which originated in the weathering of rocks, and which is the field of the life activity of immense quantities of animal and plant organisms. *By the word soil*, therefore, *we mean the loose surface strata of the earth's crust in which general dynamic processes* (weathering, erosion, etc.) *have taken place, and are taking place in conjunction with chemico-biological processes* (such as the action of plants and animals, their decomposition, the activity of microorganisms, the accumulation of humus, and the like).

Soil, according to the expression used by Prof. Docuchaiev, is a mirror which exactly reflects the mutual activities of the above-mentioned factors in its development (i.e. rocks, organisms, physico-geographical conditions, and the age of the country). Soil is the result of the factors enumerated, and if these factors are equal then the resultant soils will be equal, and *vice versa*.

Keeping in mind this fundamental statement, it is absolutely necessary, in forming a scientific classification of soils, to fix those combinations of the factors of development which cause the process of soil formation to take a certain direction, and which, generally speaking, will finally lead to equal results. We know, for instance, that only weathering of rocks can thoroughly obliterate the difference among them. This equalization of those products of the weathering of the different rocks will be still greater when the organisms which settle in them work in the same direction.

Thus it is possible, for instance, to determine what combination of factors of development will have as a result the so-called Black soil (tchernozem of Russia, and the black soil of the prairies in the United States). The most important characteristic of these soils is the increase of humus, under the influence of a temperate climate, grassy steppe plants, and lime. Under such conditions we always find black soil, as, for example, in Southern Russia, Hungary, N. and S. Dakota, Nebraska, Kansas and some other central States of North America.

If we have glacial material as the formative strata in a moderately cold climate with a large amount of rainfall, and tree vegetation, then the development of sour humus is the most characteristic feature of the soil formation. The solution of humic acids removes the greater part of the soluble matters of the soil from the upper layer, and deposits them in the lower, and as a consequence the percentage of insoluble

material (silica) increases in the upper strata. Soils of this character are not only to be found in the glacial deposits of the northern parts of European Russia, but also cover the plains of Denmark, Northern and Central Germany, Holland, and France. One may infer that this kind of soils is to be found in North America (Canada), just as it is found in the Asiatic possessions of Russia (Siberia).

The study of the typical combinations of the factors of soil formation enabled Prof. Sibirtzev to establish the following types, which include every possible variety of soil.

I. *Laterite soils.* These are developed in humid tropical climates. The characteristic feature of their development is a rapid disintegration of the formative rocks (granite and others) owing to excessive heat and moisture. Hence the salts of potash, soda, calcium, and magnesium are removed, and the percentage of iron and alumina salts in the remaining mass increases. Soils of this description are best studied in India, Ceylon, Japan, China, in parts of Russia (on the south-eastern shore of the Black Sea), and may also be found in Central America, and in Africa.

II. *Wind-blown Loess soils.* These are developed under the influence of a dry and warm climate, i.e. in the central parts of large continents (e.g. Asia). The characteristic feature of disintegration of rocks under such conditions is the formation of dust. This dust is carried by the wind great distances, and, being deposited, forms a mass of loess which is sometimes a thousand feet deep, according to Richthofen's "China." These soils are distinguished by an abundance of carbonates of calcium and magnesium, and a small amount of humus. They cover immense areas of Asia (China, Turkestan, Persia), and of Northern Africa and America.

III. *Soils of the dry Steppe.* The characteristic climatic feature is a small amount of rainfall (10—15 in.) which occurs as a rule during the summer, xerophile plants, and an alkaline character of the formative strata. To this class belong the soils of the desert and of the Cactus and Artemisian Steppe. They contain a small amount of humus, and the process of washing-out is very slow, owing to the small amount of rainfall. They are found in South-eastern Europe, Russia, Siberia, in parts of Spain (desiertos), western States of America (Utah, Nevada, Arizona, California) and in South America.

IV. *Black soils (Tchernozem).* They cover generally the grassy steppe or prairies of the temperate zone. The subsoil is mostly fine-grained, wind-blown and glacial loess, which contains a large amount

of carbonate of lime. Topography, plains; climate, continental, dry, with from 18—20 in. of rainfall, mostly in summer. The characteristic feature of this soil is an increase of neutral humus up to 15 per cent. and even more. The character of these soils may vary within wide limits, according to the conditions of a particular locality.

The Black soils are best studied in European Russia where they represent one of the best and most important soil regions. They are further found in Western Siberia, Hungary (pusstas), Bulgaria and Galicia. In the States of N. and S. Dakota, Nebraska, Kansas, Arkansas, Indiana, Oklahoma, Mississippi, and Texas the Black soil is quite analogous to the tchernozem of Southern Russia. The same soil is also found in Argentina (Buenos Ayres, Santa Fe, etc.).

V. *Gray soils* (forest-steppe soils) are developed under conditions very similar to those of the Black soils, but instead of grasses we generally find forests of deciduous trees. They have practically the same formative strata as the Black soil, but a greater amount of rainfall makes them more leached, and these conditions make possible the growth of forests instead of grass. Besides the smaller quantity of humus, which is shown by the lighter colour (gray), as compared with the tchernozem, the characteristic features of these soils are a smaller amount of calcium and magnesium, and in general of all nutritive substances which can be washed away. They are found in strips along the northern boundary of the tchernozem in Russia, Western Europe, and in North America on the boundary between prairies and forests.

VI. *Peat and ashy (Podzol) soils.* These originate in cold-temperate or cold climates, with a large amount of rainfall and little evaporation. The formative strata generally consist of glacial deposits (loam, clay, and sand). The vegetation consists for the most part of forests, heather, meadow and swamp plants. The most characteristic feature of the Podzol, owing to the development of humic acids by decomposition of organic matter, is the dissolution and removal of soluble parts of silicates (Ca, Mg, K, Na, Fe, Al), and an increase in the percentage of insoluble silica. This silica gives the soil its characteristic whiteness and ash-like texture, from which it receives its Russian name. The amount of humus in these soils is very small (2—3 per cent.), and a considerable part of it is soluble in water. These soils differ widely in character. They cover the northern parts of Europe, Russia, Siberia, and all plains of North-western Europe, and may be expected in Canada.

VII. *Fenland (Tundra) soils.* These are developed from different

kinds of formative strata which were slightly decomposed under the influence of scanty fen plants and an extremely cold climate with a large amount of rainfall. The subsoil is almost constantly frozen. Owing to the small amount of vegetation (mosses, lichens), and the insufficiency of heat, which in turn hinders the decomposition of organic matter, these soils are poor in humus. They are found in the Arctic regions of Europe, Asia, and America.

The seven fundamental groups of '*zonal*' *soils* just enumerated are spread over the surface of large continents in zones which coincide with the physico-geographical zones of those continents. The most typical expression of these zones of soils is found on the Eurasian continent; it must, however, be understood that this zonal distribution of soils is merely a rough scheme. Some factors in soil formation undergo sharp changes within comparatively short distances, and therefore we never find zonal soils covering a continent in an uninterrupted belt. In this scheme the soils enumerated are spread over the Eurasian continent beginning with laterite soils in the extreme south, and gradually reaching the tundra (fen) soils of the north.

Since the climatic factors at work will also vary with the height above sea-level we may observe as in the Caucasus a vertical zonality of soils corresponding to the lateral zonality previously described. In all these zones also certain interzonal soils are sure to occur, which owe their peculiarities to special conditions of topography alone. Such are swamp and alkali soils and the calcareous soils resulting from the decomposition of limestone. Incomplete soils of coarse materials, such as are found in mountainous regions, and alluvial soils are also common to all the zones. Furthermore within the limits of any one zone various intermediate stages are possible which depend upon the degree of intensity in the factors in their development. Such soils constitute the six (VIII—XIII) interzonal types enumerated by Sibirtzev.

If he keeps in mind this main idea in the study of the soils of a given locality, the problem of the investigator will resolve itself first of all into the determination of the factors of soil formation. This determination will show him the most important types of soils. The next step will be a more detailed study of the dynamics of the process of soil formation, i.e. how some factors of the development of soils are reflected in their character: what is the influence of the duration of the soil formation, climate, topography, plant and animal organisms, etc. When this combination between soils and the factors of their formation has become clear in the mind of the investigator, he will

know that under certain physico-geographical conditions, and from certain rocks, only certain soils can be expected, and under other combinations, other soils.

I have endeavoured to state the most important features of the genetic classification of soils which have been applied to the best classification which up to the present time has been devised for the soils of European Russia (that of Prof. Sibirtzev). In spite of its seeming complexity, its fundamental feature is the general statement that soil is the product of the conditions of its development, and that the peculiarities of soils are closely interrelated.

August, 1908.
BERKELEY, CAL., U.S.A.

ON THE INHERITANCE OF STRENGTH IN WHEAT.

By R. H. BIFFEN, M.A.

Professor of Agricultural Botany in the University of Cambridge.

The experiments carried out by the Home Grown Wheat Committee of the National Association of British and Irish Millers have demonstrated the important fact that certain varieties of wheat retain their strength under our conditions. One of the best known of these varieties is Red Fife, the grain of which forms the basis of the graded wheat known as Manitoba Hard. Red Fife has now been tested in this country for six seasons and its strength is still found to be equal to that of the same variety when grown in Canada. Further a somewhat impure stock of the same variety has been cultivated in the Midlands for some sixteen seasons without showing any signs of diminution in strength. As grain of this character is considerably more valuable than that generally grown in this country and as there is a steadily increasing demand for it, the Committee recommended the experimental cultivation of Red Fife in various parts of the country. The results of these trials have shown that in some localities Red Fife will produce more profitable crops than any of the common English wheats, though in others its yield is too small for its profitable cultivation. In spite of very complete returns furnished by growers in most parts of the country it seems impossible at present to state with any precision what the factors are which determine whether it can be grown successfully. Its extended cultivation has also brought out the fact that the straw is not as rigid as the farmer would wish it to be and more than one grower has condemned the variety on this account.

Concurrently with these investigations the attempt has been made to build up a variety possessing the high cropping capacity of the

common English wheats and the strength of such a variety as Red Fife. To make any definite progress with this work information as to the mode of inheritance of strength was essential. At this time, although wheat breeding experiments had been carried out on an extensive scale in Canada, the United States and Australia, no information on this point was available and the problem had to be attacked from the beginning.

Unfortunately strength and weakness do not form a pair of sharply differentiated characters, and at times it is exceedingly difficult to say from a simple inspection of the grain whether it is strong or weak. In fact, at present, the one absolute criterion is to convert the grain into flour and test it in the bakehouse—a proceeding which is obviously impossible where small samples only are available. It is probable however that in the near future the breeder will have other methods at his disposal for differentiating between these characters[1]. Failing these, certain rough and ready tests have been used in the earlier stages of the work which, as the sequel will show, have given satisfactory results.

As a general rule strong varieties of the bread-making wheats have a semi-translucent grain, and this appearance taken in conjunction with minor characteristics such as bloom, colour and hardness forms the basis on which wheat is valued in the markets. Weak wheats on the contrary have opaque or starchy grain. Too much stress must not be laid upon this difference for mere translucency will not differentiate with certainty between varying degrees of strength, and it must be recognised that a wheat expert's judgement is, though often perhaps unconsciously, largely influenced by a familiarity with the actual variety of the wheat and a knowledge of its baking properties derived from previous experience. A complication is also introduced by the fact, which is not generally recognised, that translucency is largely determined by the conditions under which a wheat is grown. Thus it is by no means unusual to see samples of such a weak wheat as Squarehead's Master with grain as translucent as that of a good sample of Red Fife. Such a sample brings higher prices in the market than those with opaque and starchy-looking grain though it is questionable whether from the point of view of strength only it is intrinsically more valuable. If the grain is tested in the bakehouse there is little difference between the starchy-looking weak grain and that which has some of the appearance of strong wheat. These translucent weak wheats never appear to acquire the hardness of really strong wheats and consequently it has become a common practice amongst buyers to test

[1] Wood, *Journ. Agric. Sci.* Vol. II. p. 139.

the hardness of the grain by biting it. A useful modification of this process in the laboratory is to crush a few grains of the wheat to be tested on an iron plate. The weak grain then breaks to a fine soft powder whilst the strong grain crushes to angular fragments or to a gritty powder. How far this test is generally applicable I cannot say, though with the varieties of wheat with which these experiments have been carried out it has given useful results. A third and certainly the most valuable method of testing wheats, particularly where a comparison is to be made between such strong varieties as Red Fife and our ordinary English wheats, is to chew some twenty or thirty grains until the starch and the grain coats have disappeared. A weak wheat so treated leaves a small quantity of soft, often slightly viscid gluten in the mouth, whilst a strong wheat leaves far more gluten which can be stretched into long threads without breaking or will readily return to its former shape if a small ball of it is pressed flat. This chewing test will differentiate between all of the common English wheats and the strong Fifes with certainty, though it is difficult to be certain of the results if varieties closely approaching one another in strength have to be classified. The drawbacks to its use are obvious.

In the earlier experiments, when it was desirable to know with as great accuracy as possible the results of crossing strong and weak varieties together, the attempt was made to circumvent the difficulties caused by the appearance of the grain by growing as the weak parents varieties which previous experience had shown produced starchy-looking grains only. The most suitable for the purpose have been Rough Chaff and Rivet wheat. These grown on comparatively poor and unmanured soil year by year have rarely produced any translucent grains. Even in these cases a more ample supply of food materials, as for instance when the plants were grown in garden soil, altered the appearance of the grain considerably. In the later experiments, where it has been necessary to employ such varieties as Squarehead's Master, Browick, Stand Up, etc., opaque and translucent grains have often been found in one and the same ear when the varieties were grown in poor soil. In such cases no sustained attempts have been made to trace the inheritance of strength in detail but the strongest-looking grain of the F. 2 plants has been picked out and tested by chewing before further propagation. The varieties used as strong parents have always under our experimental conditions produced translucent grain, though in more extensive field trials opaque grains have been far from rare. The occurrence of such grain at this stage does not offer any particular difficulty, as practice

enables one to distinguish between weak and weak-looking strong grain with considerable certainty, and one can always fall back on the chewing test. Red Fife has been used more than any other variety as a strong parent, but a considerable number of other varieties isolated from graded Russian and Canadian wheats by Mr A. E. Humphries have been included in the experiments. Certain of these have given valuable statistic results, but as the varieties themselves have not proved as strong as Fife or its hybrids, their further employment except for special purposes has been abandoned.

The hybrid grains resulting from a cross between strong and weak varieties have the appearance of strong wheats, though in view of the fact that they were generally small and shrivelled, and as they were required for sowing, supplementary tests could not be applied to them, this appearance might possibly be misleading.

The F. 1 plant-generation raised from the hybrid grains was grown under favourable soil and space conditions in order to secure as large a yield as possible. All of the plants produced grain of undoubted strength—matching as far as such matters can be determined the strong parents in this respect. No indications of segregation into strong and weak grains could be detected at this stage.

The F. 2 generation was then grown under uniform soil conditions and each plant harvested separately. Grain from one or more ears was rubbed out and placed in a watch-glass standing on a white background. When a series of a hundred plants had been so treated the grain types were sorted out, using samples of the grain produced by the descendants of the original parents for comparison. By growing the parent plants on differences in appearance due to the ageing of the original stock and to climatic conditions can of course be avoided, and further a sufficient supply of the parent wheats can readily be obtained for the more critical comparative tests of the bakehouse. In all of the 32 cases so examined the segregation into strong and weak types was perfectly obvious, and no difficulty was experienced in picking out samples the exact counterpart of the parents as far as strength was concerned, this being estimated by each of the three methods already described. On attempting however to count out the types represented in the F. 2 generation it was found that some crosses gave simple Mendelian ratios whilst others could not be sorted with any accuracy. An example of the first case is afforded by a cross between Rough Chaff and Fife wheat which has already been partially described[1]. Four types of grain were present, namely strong

[1] Biffen, *Journ. Agric. Sci.* Vol. I. p. 39.

red, strong white, weak red and weak white: in the first hundred samples the proportions for these were 58 : 16 :: 18 : 8, in the second 59 : 18 :: 16 : 7, showing a reasonably near approach to the 9 : 3 :: 3 : 1 ratio expected where the two pairs of characters, redness and whiteness, and strength and weakness, are concerned. Similar results have been obtained where Rough Chaff has been crossed with strong Russian wheats. As an example of the second class of cases a cross between Red Lammas and Red Fife may be mentioned. The F. 2 generation contained plants with obviously strong and weak grains, but between the extremes there was a long series of plants which could not be classified with any certainty. In some of the grain samples a few starchy flecks occurred amongst grains which otherwise appeared to have great strength, whilst in other samples there was a flecking of glutenous patches on a starchy ground[1]. One series of plants gave a ratio of 23 completely starchy to 92 with more or less glutenous grain. The latter group undoubtedly contained weak-grained plants, for the weak parent grown on the same plot produced grain of this nondescript type. The chewing test was then resorted to and this further indicated that the series contained many plants of strength intermediate between that of the parents, though the actual classification into strong, intermediate and weak seemed to be impossible.

A number of cultures, each containing some thirty to forty plants, were raised from individuals of the F. 2 generation, these being further selected, for the most part, for recessive chaff and colour characters. Confining our attention to the grain characters the resulting F. 3 generation gave the following results:

Rough Chaff × Fife:	
F. 2 plants sown	Types of F. 3
Strong 50	Strong 16, mixed 34
Weak 12	Weak 12
Lammas × Fife:	
F. 2 plants sown	Types of F. 3
Strong 50	Strong 50
Flecked 20	Strong 3, mixed 17
Weak 10	Weak 10

In the first case then strength is dominant to lack of strength and the heterozygotes are indistinguishable from the dominant homozygotes. In the second case the dominance is not so sharply marked and the

[1] The weak parent frequently shows this same flecking of the grain.

heterozygous individuals can be distinguished with a certain degree of accuracy.

These examples are typical of some thirty others, though in the majority of these it was not considered necessary to determine whether the recessive lack of strength was pure from the moment of its appearance in the F. 2 generation. Forty of the sixty-six strong cultures proved homozygous in all respects. Sufficient grain from these was available to plant plots of one-fortieth to one-twentieth of an acre in extent. In order to multiply the stock rapidly the grain was planted at four-inch intervals in drills eight inches apart and the whole set of plots was surrounded by a belt of Squarehead's Master and Browick wheat to prevent, as far as possible, depredations of sparrows. The series formed a striking demonstration of the value of Mendelian methods to the plant-breeder, for in this, the F. 4 generation, they were, as far as the eye could see, perfectly fixed. The only rogueing required was to pick out a few plants of Squarehead's Master which had found their way in whilst the protecting crop was being drilled. At this stage it was possible to obtain some idea as to how far each type was suitable for further cultivation, and by harvest time it was decided to retain six of the forty for another season's test. As there was a sufficient quantity of grain produced on some of the plots to mill and bake, a number were chosen at hazard and harvested. Owing to the pressure of work at this time of the year they were not cut until some ten days or a fortnight after the normal time of harvesting. Further the quantity was too small to put into a rick so that the grain had no opportunity of finishing in the stack. Instead it was sent directly to Mr A. E. Humphries of Coxes Lock Mill and ground and baked within a few weeks of cutting. There were thus two factors which the baker would consider depreciatory to strength, namely over-ripeness and lack of ageing.

In the milling operations it was soon evident that strong wheats were being dealt with, for the man in charge of the operations noted that the grain ground like Manitoban wheat. When one considers that no information had been given as to the origin of the wheat and that one of the parents, Red Fife, is the chief constituent of the graded Manitoba Hard the observation becomes a striking one. The flour was then handed to the baker who under the direction of Mr Humphries tests the wheats ground in the ordinary course of business in the mill and also the numerous varieties grown by the Home Grown Wheat Committee. For the methods of testing the reports of this Committee should be consulted[1]. Here it is sufficient to say that half-quartern

[1] Report of the Home Grown Wheat Committee, 1905.

loaves of the cottage shape are made under ordinary baking conditions, as tin-loaves and the small bun-like loaves in favour amongst continental workers are not considered satisfactory. For purposes of comparison a batch of loaves made from flour of known strength is baked at the same time. By using these as a standard the trained baker can express his opinion of the flours under test by means of a series of marks. The baker's object is to secure the best results the flour is capable of producing, as would be the case in ordinary commercial working. He is at liberty to use his judgement as to what conditions will give him this result, so that no single factor is necessarily constant throughout these trials. Where an abundant supply of flour is available the baker determines in the course of a few tests the conditions which will give him the best loaves, but where, as in the case of these hybrids, the maximum amount at his disposal was some thirty pounds of grain, there is obviously a tendency for the values to be lower than they would in practice.

The marking is also arbitrary and conveys little information to those not actually familiar with the details of these experiments.

In addition to these varieties others have been tested in the two following years. In each case one of the parents was a strong wheat with a baking value expressed by marks ranging from 80—90. The second parents were average English wheats marked at their best as high as 60 but often with values considerably lower than this. For instance a good sample of one grown under the same conditions as the hybrids only obtained 45 marks.

Throughout the baking operations it was evident that the hybrid varieties had very different properties from those of ordinary English wheats. The shortness, characteristic of the dough of the latter, and the heaviness of the loaf were missing, and the finished product showed a marked resemblance to loaves of Red Fife wheat. The values assigned to the different varieties were as follows:

88, 86, 86, 84, 84, 84, 83, 83, 80, 80, 80 and 70.

The baking tests thus confirm the conclusion, previously arrived at by simply inspecting or by chewing the grain, that strength and lack of strength segregate from one another in the F. 2 generation in the same manner as the morphological characters already examined. Further these primitive methods of judging the quality of grain have proved more reliable than was to be expected. Assuming that they are generally satisfactory, then it may be stated that in all of the

crosses hitherto examined strength has been inherited in its entirety. Where for instance the value of this character in the parent is expressed by the mark of 75 or 95, then individuals showing a strength of 75 or 95 can be isolated in the F. 2 generation and obtained in a stable condition. Whilst the majority of the hybrids raised later in the course of this investigation have not reached the stage at which reliable baking tests can be made, the results obtained with those that have agree satisfactorily with this hypothesis.

Against it may be urged the fact that one of the twelve varieties tested differs considerably from the others, the marking of 70 being very low, even when the errors inseparable from baking trials are taken into account. This wheat however proved to be heterozygous with regard to strength and in the following season undoubtedly weak wheats were raised from it.

In the hope that the total nitrogen content of wheat grain would prove a reliable index to its strength and so save much labour in growing sufficient quantities of a large number of new varieties to test in the bakehouse, some preliminary investigations into the inheritance of this character have been made. It appears to be a general characteristic of strong wheats that they contain a higher total nitrogen percentage than weak wheats when grown under the same conditions. This proviso is necessary, for by growing a weak wheat in rich soils it is possible to give it a higher nitrogen content than a strong wheat grown under conditions where it cannot obtain so abundant a supply of food materials. A further complication is introduced by the fact that there may be great differences between ears from the same plant. Thus one large coarsely grown plant of Squarehead's Master examined ear by ear showed a range of 2·6 to 1·8 per cent. of nitrogen, the ears with the highest percentage being those side tillers which had failed to mature properly. To minimize the errors due to these sources the grains have been thickly sown at uniform distances, namely two inches between the grains and six inches between the rows. Under such conditions only two or three tillers are produced on each plant and in these the nitrogen contents have proved to be approximately the same. On harvesting the two outermost plants of each row have been rejected as they would obviously have had a far greater root-range and supply of food material than those surrounded by other plants.

The F. 2 generation chosen for examination was that of a cross between *Triticum polonicum* (Polish wheat) and *T. turgidum* (Rivet wheat). The Polish wheat has an unusually high nitrogen content

and the grain is brittle, translucent and white in colour. Rivet wheat on the other hand is a typical starchy, red wheat with a soft mellow endosperm and low in nitrogen. The average nitrogen contents are 2·2 and 1·6 per cent. respectively.

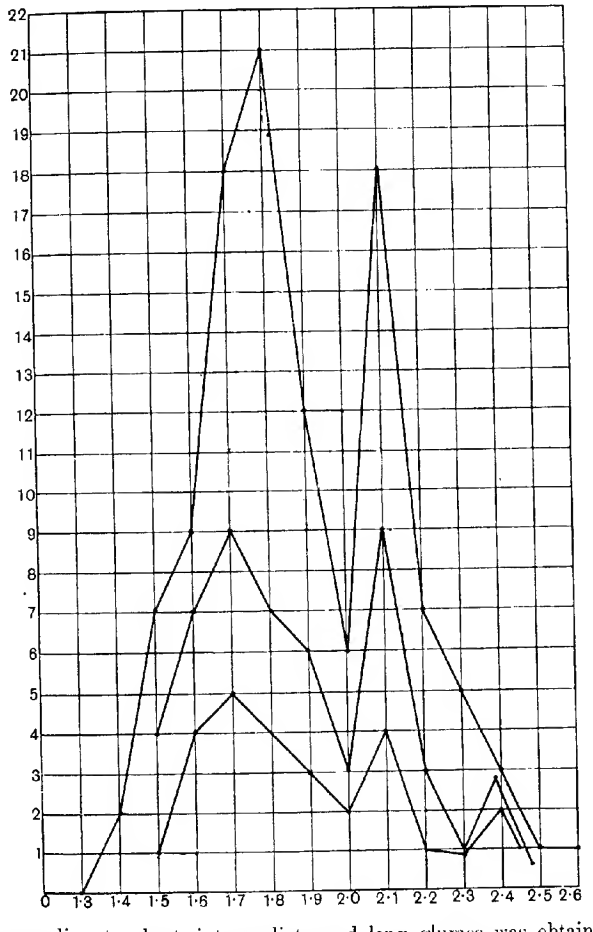
In the F. 1 generation the grain was uniformly brittle, translucent and red. The nitrogen content was thus probably high, but as the plants were grown at wide intervals any determinations of its value would have been misleading.

In the following generation the expected segregation into red and white, translucent and starchy occurred, but the mode was not as simple as in certain of the crosses already described. The proportions of red grained plants to white and also the characters provided by the shape of the grains still require to be investigated in more detail. Confining our attention to the endosperm characters, the most obvious feature was the presence of many plants with grain which could not satisfactorily be placed by eye in either the starchy or the translucent classes. The fact was unexpected, for the parents always retain their brittle translucent or their starchy mellow characteristics under our conditions. Before determining the total nitrogen contents ears from 110 plants were sorted into three groups, namely those with either long or short glumes similar to those of the parents[1] and those with glumes of an intermediate length. The grain was then rubbed out from the central portion of each spike and the nitrogen determined by Kjeldahl's method. The remaining grain was kept for reference and future sowing. The results of the analyses are shown in the following curves. The lowest curve shows the distribution of the nitrogen contents over the long-glumed individuals, the one above it the long- and short-glumed and the uppermost the three groups taken together. From the general similarity of these curves it is evident that the distribution of the nitrogen contents is not affected by the shape of the glumes, for it is practically uniform for each of the groups. The curves however do not show sharp segregation into two groups, one corresponding with the nitrogen content of Rivet wheat (1·6) and the other with that of Polish wheat (2·2). There are however two distinct summits in the curve at 1·8 and 2·1 suggesting that it is built up of two or more partially overlapping curves of error.

The failure to give sharp segregation into two or more groups in the F. 2 generation is not uncommon in crosses between cereals. In the case

[1] Biffen, *Journ. Agric. Sci.* Vol. I. p. 37.

of this same cross for instance it seemed impossible to separate the various glume lengths into distinct groups, yet when a series of measurements was made it was found that three sharply discontinuous curves



corresponding to short, intermediate and long glumes was obtained[1]. A further generation raised to test this point showed, with no exception

[1] Biffen, *Journ. Agric. Sci.* Vol. I. p. 37.

amongst the hundreds of individuals taken from the F. 2 generation, that all falling within the limits of each of the curves bred true either to the short- or long-glume character or when chosen from the intermediate series they proved heterozygous. In other cases a partial overlapping of the component curves has tended to obscure the results. This is particularly common where the length of the internodes of the ear is investigated, as for instance in some barley hybrids[1]. An analysis of the F. 3 generation is then required to unravel the mode of segregation, when it is found that the heterozygotes can be at once distinguished from the homozygotes and that they occur in the ordinary Mendelian proportions.

The difficulty then is to say whether a plant with a nitrogen content of say 1·7 per cent. is an extracted low nitrogen type or a heterozygous plant with a lower content than the mean of the series. To give a complete proof it would be necessary to grow on each of the individuals of known nitrogen content and determine that of each of the plants produced from them. Such an experiment was impracticable at the time and consequently an approximate analysis has been attempted by comparing the appearance of the grain of the F. 2 parents with their descendants. It was believed that this could be relied upon to give a sufficiently accurate result because the analyses of the F. 2 generation showed that grains with a translucent endosperm were high, and those with a mellow starchy endosperm were low in total nitrogen content. Grains which were flecked with starchy patches generally showed an intermediate nitrogen content but the percentages varied widely from what one would have expected from the extent to which the grains were flecked with starchy patches. A slightly flecked sample for instance did not always show a higher content than one in which the greater part of the grains were starchy.

At harvest time bunches were made containing an ear from each of the descendants of all of the F. 2 plants of known nitrogen content, and later the grain was rubbed out from the bunches. Those producing translucent grain only were considered to be homozygous with regard to a high nitrogen content, and similarly those with mellow grain were considered to be pure with respect to a low nitrogen content. Analyses made from these bulk samples gave an average content of 2·4 and 1·6 per cent. respectively, figures which compare satisfactorily with those of the original parents. The remaining samples were mixtures

[1] *Journ. Agric. Sci.* Vol. II. p. 201.

of translucent, starchy and flecked grains, the last type being the most abundant. The occurrence in one F. 3 bunch of grain resembling each of the parental forms shows that the F. 2 plant from which it was descended was heterozygous in its endosperm characters. The total nitrogen contents of the majority of such F. 2 plants fell within the limits of 1·7 and 2·1 per cent., the three exceptional cases having percentages of 1·5, 1·6 and 2·2 respectively. Thus a nitrogen content of from 1·3 to 1·6 or from 2·2 to 2·6 marked with approximate accuracy the pure low or pure high types which could be distinguished from the heterozygotes by analysis only.

The total number of plants with a low nitrogen content was 26, 63 appear to be heterozygotes and 21 were high in nitrogen. The figures are far from the expected ratio of 1 : 2 : 1. How far this is due to the errors incidental to such an investigation is uncertain and the possibility must not be overlooked that a more complex mode of segregation may exist here. It is however sufficiently clear that the breeder can rely on the segregation of the differing nitrogen percentages and isolate them in the F. 3 generation with no particular difficulty[1].

The baking properties of a number of hybrid wheats have recently been investigated by C. E. Saunders. In summing up the results he states that "while it is no doubt possible that in some cases a cross bred wheat may possess baking qualities the same as one of the parents, the results given here seem to show conclusively that baking strength is not a Mendelian character, that is to say, is not always inherited from one or the other parent in a pure condition[2]." From an analysis of the data provided in this paper it appears that the cases where no Mendelian segregation is shown are provided by the varieties Percy, Preston, Huron and Stanley and also certain selections from them. A brief account of the history of these varieties will be sufficient to show how little reliance can be placed upon the evidence. The whole series results from crosses made in the year 1888 between Red or White Fife and Ladoga. They were bred true to type by discarding all the variations produced. This process appears to have been completed about the year 1893, for in subsequent years the records for their yields are published in the annual reports of the Experimental Station. In 1903 the varieties were submitted to milling experts whose reports

[1] The analyses were made by Mr S. F. Armstrong of the Agricultural Department of Cambridge University.

[2] Saunders, Central Experimental Farms, *Report for* 1907, p. 35.

make it evident that they possess strength of the same order as that of Fife. At the same time a detailed examination of their properties was made by the chemist attached to the Experimental Station. Again there was no question about the great strength of these varieties, for on classifying them Percy was placed at the head of the list, Stanley was found to be the equal of Red Fife, whilst Preston was slightly inferior[1]. Further Preston was tested at Minnesota about this time and in the American report is the statement that it "bids fair to be a strong rival of our best Fife and Bluestem wheats[2]." More conclusive proof of the fact that these varieties once possessed the strength of Fife it would be difficult to find. Yet the results of the baking tests carried out on the crops of 1905 and 1906 show that they are considerably inferior to the Fife wheats. In the meanwhile though, the discovery was made that these wheats were not absolutely true to type. In the 1903 report is the following statement:—"The tendency for cross-bred cereals to vary for a number of years after their production is also seen in the case of those varieties produced at the Experimental Farm in the earlier days of its history. Some of these such as Preston wheat, Stanley wheat, etc. have already attracted a good deal of attention. It is found however that each of these as now grown is not of one fixed type, but contains a small proportion of kernels which appear foreign. Efforts are being made to improve these varieties by reducing each of them to one type as quickly as possible. Descriptions of the varieties will be published when the types are decided upon and fixed." At this stage the information begins to fail and we are not told whether Mendelian methods were employed to secure fixity of type. Had the attempt been made to isolate individuals homozygous as far as strength was concerned, it would probably have been mentioned. As it is not, one is forced to the conclusion that no attention was paid to this point and that the pre-Mendelian methods, so well illustrated in subsequent reports, were considered sufficient for fixing so elusive a feature as strength.

A number of the cases investigated by Saunders would appear to show that strength is inherited in its entirety. Certain of these may be quoted though it would be unwise to lay great stress on them at this stage, for we are unable to compare the strength of the hybrids with that of the actual parent plants, a feature of more than ordinary importance where Red Fife is concerned.

[1] Saunders, Central Experimental Farms, *Report for* 1903, p. 21.

[2] Univ. Minnesota, *Bull.* 62, p. 362.

The majority of these cross-breds have Fife as one parent and the most one can do is to compare the hybrids with ordinary Red Fife. This is doubly unfortunate, for Saunders has brought forward evidence which seems to show that Fife can be split up into strains showing an appreciable difference in strength. In other words there may have been considerable differences in the strength of the Fife plants used as parents. Further Saunders has shown that it is frequently far from pure and contains, as a mixture, several varieties of wheat some of which are strikingly similar to it in external characters though not in strength. These stray plants might seriously prejudice the accuracy of any breeding experiment, for, when in the stage at which pollination has to be performed, it must be well-nigh impossible to distinguish them from Fife itself. Failing this evidence we must assume that the strength of the plants used as parents fell somewhere between the limits Saunders finds for Fife. The tables show a range of 107 to 89. The two extreme figures may however be neglected, for the mark of 107 is due to the ageing of the flour and 89 to growth under unfavourable conditions. This leaves a range of 102 to 95 which probably represents with sufficient accuracy the strength of Red Fife. Hybrids marked between these limits may then be said to have the strength of Fife.

Thus Gatineau, a variety resulting from a cross between Red Fife and Goose wheat, shows the strength of Red Fife (96) whereas Goose wheat is marked at 81. Outlook (99) further shows the strength of its parent Fife, but that of its second parent, Rideau, is not given. Chelsea and Marquis, marked respectively at 99 and 98, are also, in all probability, Fife crosses since the general plan of these experiments is to obtain early ripening associated with the strength of the Fife wheats and "both resemble Fife rather closely but are distinctly earlier in ripening."

In these four cases the agreement between the strength of the hybrids and of the Fife parents is little less than extraordinary when one takes into consideration the numerous sources of error present in all baking trials.

The only other cases which have come under my observation, where strong and weak wheats have been crossed together, are those provided by the late W. Farrer's experiments carried out in New South Wales. After observing the segregation into strong and weak in the F. 2 generation of the cross between Rough Chaff and Red Fife, I wrote to Farrer asking whether he could check the result on the crosses

between Red Fife and Indian wheats which he was raising at the time. In his reply he told me that in almost all the crosses he had one of the parents was an unfixed cross-bred, generally of the first generation. Accompanying the letter were parents and a long series of these hybrids, some at the F. 2 stage, others one or more generations older. The weak Indian wheats under the dry Australian conditions produced grain which, compared with our own wheats, seemed unusually soft and starchy, whilst the Fife grain was as hard and translucent as any I have yet seen. With this pronounced difference in appearance as was only to be expected the segregation was exceedingly sharp and every sample could be graded definitely into one of two classes; it was either strong or weak. Farrer's experience of using Fife as a parent was that it gave him hybrids as strong as Fife itself. As an example he quoted the case of a variety he had named Comeback which at that date was used in some of the local bakehouses and giving results "as satisfactory as the Manitoba flour." Since then many of Farrer's hybrids have been obtained in sufficient quantity for field culture and baking trials. Guthrie in a recent monograph on the wheats of New South Wales[1] has given descriptions of some of these and in his account of Farrer's work he confirms the statements I have quoted above. He states that "the Fife Indian combinations are particularly promising ones and are pretty certain to become extensively grown in the near future. These cross-bred wheats of Mr Farrer's produce flour which is quite as strong as the Fife wheats and they are more suitable than these for cultivation under our conditions."

The variety Comeback has been grown this season (1908) for the Home Grown Wheat Committee, and it is interesting to note that under our climatic conditions as well it shows the great strength of its parent Fife.

These results, taken in conjunction with the experiments described above, show that the problem of breeding strong wheats suitable for English conditions should offer no especial difficulties. The question however has been repeatedly raised as to whether it is possible to grow sufficiently large crops per acre of such wheats, the assumption being freely made that the capacity to yield large crops is associated with the production of starchy grain. To give a decided answer requires a knowledge of the mode of inheritance of high- and low-yielding capacities, assuming that these form a pair of Mendelian characters.

[1] Guthrie, *New South Wales Agric. Gaz.* Vol. XVII. p. 1173.

The evidence for and against this view is at present too fragmentary to discuss and the only data we have at our disposal are provided by the yields of the strong-grained hybrids raised in the course of these experiments. Certain of these have now been grown in field plots ranging from one-fortieth of an acre in the first to two and a half acres in the third season, so that the measures of their cropping capacity are known with approximate accuracy. These varieties were chosen at the F. 4 stage for high-yielding capacity, those giving no promise of satisfactory crops being rejected. At this period the seed-rate was far below the normal. The plants tillered well especially at the margins of the plots and yielded heavily, the crops ranging from 42 to 30 bushels per acre. However the areas were small and owing to shortage of grain there was no possibility of duplicating the plots, so that these determinations are of little value. In the following season plots of these varieties were drilled at the rate of 3 pecks of grain per acre. They gave an average yield of 35 bushels. A repetition of the test this season (1908) with ordinary seed-rates gave yields of between 32 and 33 bushels. A mean yield of 33 bushels per acre probably gives the cropping capacity with fair accuracy. Comparing this with the parents the Red Lammas and Rough Chaff yield at the rate of about 33 bushels and Red Fife 20 bushels per acre.

Thus there can be little doubt that high-yielding capacity and strength can be obtained in combination in the same variety, though whether high- and low-yielding capacity segregate at the F. 2 stage in the ordinary fashion remains to be determined.

ON A METHOD OF CHECKING PARASITIC DISEASES IN PLANTS.

By M. C. POTTER, M.A.

Professor of Botany, Armstrong College, Newcastle-upon-Tyne.

ALTHOUGH the study of the action of Bacteria in pathogenic relation to the higher plants may be described as in its infancy, a great deal of sound work has been done in this branch of Phytopathology, and various types of bacterial diseases have been definitely established.

One large class comprises those in which the Bacteria invade the cells of the parenchyma, producing a rapid degeneration of the cell and its contents and complete destruction of the tissues. To this group belongs the "White-rot" of the Turnip, caused by *Pseudomonas destructans* (Potter)[1]. In a previous account of the disease the author showed that this Bacterium owed its power of attacking living plant-tissues to the fact that it is able to secrete an enzyme (cytase) which acts upon cellulose, dissolving the middle lamella, and effecting a swelling and disintegration of the cell-wall; while at the same time it produces a toxin fatal to the protoplasm of its host-cell. These properties enable the Bacterium to completely destroy the plant structure. Its passage has been traced through the softened cell-wall[2], and, as its action extends rapidly from cell to cell, the entire parenchymatous tissue becomes speedily reduced to a mere watery pulp.

In the particular class of bacterial diseases falling under this category, in which the secretion of the cytase and toxin is responsible for the destruction of the cells, any attempt to check the invading organism must seek to deal with these two factors.

In working out a solution of the problem before us, the injurious effect upon any organism of an accumulation of its own waste products

[1] *Proceedings of the Royal Society*, Vol. 67. [2] *Ibid.* Vol. 70.

suggested itself as having a direct bearing upon the question. It is an admitted physiological fact that the waste products of metabolism, when permitted to accumulate beyond a certain stage, have a tendency inimical to the existence of the organism itself, gradually checking growth and activity and producing results which finally prove fatal. The yeast plant provides a convenient illustration of this point, for it is well known that the alcohol formed by its metabolic activity, if allowed to accumulate beyond a certain percentage, acts as a limiting factor preventing a continuance of the process of fermentation. An application of this principle to the checking of disease seemed to offer a curative method capable of demonstration in the laboratory.

Pseudomonas destructans, being a bacterial parasite of very virulent type and one which was found to have a rapidly destructive action upon the turnip, appeared to present a suitable subject for experiment.

In the account given of this disease it was shown that *P. destructans*, both when growing as a parasite upon the turnip and also in a nutrient solution, produced a substance toxic to the living plant cell which retained this special property after boiling; the cytase, on the contrary, was proved to be effectually destroyed when submitted to a temperature of the boiling point.

It is possible that the toxin may be one of the waste products of the bacterial metabolism, but on this point no special investigations have been made; however that may be, it is a body which can be separated from the cytase, and it is probable that a substance toxic to the protoplasm of the higher plants may, under certain conditions, prove fatal also to the life of the Bacterium.

It seemed not unreasonable then to contemplate the preparation of a solution, from which the enzymes had been eliminated, which would be toxic to the Bacteria, and when applied to the tissues under attack might effectually destroy the activity of the Bacteria and succeed in checking their ravages, thus enabling the wound to heal.

A sterile turnip broth, prepared as described in my previous paper and sown with *P. destructans* in pure culture, develops a rapid growth, and after a short time is found to be highly charged with the cytase and toxin in addition to other waste products of metabolism. Such a solution, when concentrated by evaporation under a partial vacuum, contains these bodies in a greater degree of strength and provided the medium utilized for the purposes of the present investigation.

The detailed method of experiment pursued was as follows. A litre of turnip broth was sown with a pure culture of *P. destructans* and

incubated at 30° C. for three weeks, this period of time being adopted in order to permit the Bacteria to exhaust the nutrient solution as far as possible, and to allow for the full accumulation of all waste products. At the expiration of this period measured portions of this solution, of 100 c.c. each, were reduced to 75 c.c., 50 c.c. and 25 c.c. respectively. The concentration was effected by distillation at 60° C. under a reduced pressure produced by a Sprengel's air pump. These solutions of the three degrees of concentration were drawn into a series of plugged, sterile test-tubes, each containing about 5 c.c., and these were then steamed for one hour on three consecutive days. This treatment, while destroying all enzymes, rendered the preparations completely sterile and free from contamination by other organisms.

One of each of these series of preparations was inoculated with *P. destructans* and incubated at 20° C. After an interval of some days, stab-cultures from these tubes made in turnip gelatine produced no development; while those taken at the same time from the original culture gave a vigorous growth.

The action of the prepared solution upon the living Bacteria was equally striking under the microscope. Upon examination of a fragment of turnip affected with "White-rot," it was seen to be swarming with the Bacteria moving in all directions, but when the solution was drawn under the cover-slip and it reached the Bacteria they at once became paralysed, and exhibited no further signs of activity.

It was thus evident that the prepared solution (henceforth described as the *toxic solution*) contained properties undoubtedly toxic to this Bacterium.

Trials of the effect produced upon tissues infected with *P. destructans* were made first with the most concentrated of the solutions.

A perfectly sound turnip was cut in half, and both halves were inoculated with a pure culture of *P. destructans*, and kept in the same damp chamber until the disease was fully established. One half was then treated with the toxic solution; while the other, which served as a control, was allowed to develop the normal course of the disease unchecked, though still under precisely similar conditions.

The general method of inoculation was to sow the Bacteria in a small excavation made in the centre of the blocks of turnip. After some 24 hours it was noted that the parasite had gained a strong hold, and had penetrated several cells deep into the living tissue. One half of the turnip was then selected for further experiment. A portion of the toxic solution was dropped into the inoculation cavity and allowed

to act upon the attacked tissue for about three hours, the length of time depending upon the depth to which the Bacteria had penetrated. After this time the surplus moisture was removed with blotting paper or drawn off by means of a pipette.

The inoculated spot treated in this way soon showed signs of alteration, the cells became brown and dried up, and the decay proceeded no further. In some cases a second application of the toxic solution was necessary to prevent a further extension of the bacterial action, and in rare cases a third. Three applications, however, were required only when the cells of the root in the beginning of winter had passed over into a pithy condition in which the rate of progress of the disease was more than usually rapid.

The effect of treatment with the toxic solution was remarkable; penetrating the cells and intercellular spaces, it rendered the conditions of bacterial life impossible, the decaying area did not increase and the progress of the disease was completely arrested. A striking contrast was exhibited in the untreated half: the infected spot gradually increased and the invasion spread until the whole mass became affected and the block reduced to a complete state of rottenness, becoming converted ultimately into a mere watery pulp.

Cultures extending over a longer period to allow the attack to obtain a stronger hold were equally successful. Even when two or more days were allowed for the spread of the disease and the affected area was thus enlarged to two or three centimetres in diameter, it was always possible to check any further action of the Bacteria in the manner described, while the controls entirely succumbed to the attack.

The experiments were repeated upon a number of turnips in succession, always with the same result. The two halves of the turnip were invariably placed in the same damp chamber, so that exactly similar conditions were assured. It should also be mentioned that the cleansing and drying out of the wound, described for the half treated with the toxic solution, was also performed in the case of the control and any suspicion of preferential treatment was thus avoided.

Similar experiments carried out with the weaker solutions, that is those in which the volume was diminished by evaporation to one half or three quarters only, proved to be quite effective. The Bacteria were unable to grow in these solutions, and the latter when applied to the diseased tissues prevented any further extension of the cellular destruction.

It should be stated that, as was to be expected, the toxic solution

exercised also a very injurious influence upon the healthy cells of the turnip, the protoplasm of those bordering upon the affected tissue quickly becoming brown and dead. But this influence extended only to a limited area. As the Bacteria were killed, the continued production of the cytase became impossible and the wound was able to heal over.

Microtome sections of pieces of turnip, taken from the inoculated area to which the toxic solution had been applied and fixed in Fleming's solution, presented the following appearances. The sections, stained in Heidenhain, showed externally a layer of débris composed of Bacteria and dead cells, with fragments of cell-wall; internal to this, cells with swollen and contorted walls showing separation along the middle lamella. In these cells the Bacteria occupied a large part of the cell-cavity or were present as a layer in contact with the inner surface of the cell-wall. Succeeding these again were cells in the first stages of attack, some apparently showing plasmolysis, which gradually merged into the healthy cells of the unaffected region.

This toxic solution prepared from the turnip is not necessarily toxic to other micro-organisms. *Penicillium glaucum*, *Bacillus subtilis* and *Proteus vulgaris* flourished when sown upon it, even in the solutions of greatest concentration. But even after these organisms had lived for some time in the solution, it was found to be still incapable of supporting *P. destructans*. It has been further observed that these solutions, though toxic in a high degree to the turnip cell, are less poisonous to the cells of other plants. Thus when sections of beetroot were soaked in the solutions, plasmolysis could be observed, particularly in the solutions of the highest concentration; but when placed in water the protoplasm regained its natural position in contact with the cell-walls, and it was only after immersion for a day that the escape of the red colour showed that the beetroot cells had been at last killed.

Some investigation was made as to the extension of the curative principle, as here exemplified, in other directions. A common disease of oranges and lemons is produced by *Penicillium italicum* and *P. olivaceum*, causing the destruction of the tissues as described by Wehmer[1]. These fungi grow readily upon an orange gelatine and may thus be obtained in pure culture. When grown for some time in sterilized orange juice, this medium becomes charged with waste products of metabolism which prevent any further growth of the fungus. In a similar manner a concentrated preparation of this solution will prevent the extension of the decay in oranges attacked by these fungi.

[1] *Beiträge z. Kenntnis einheimischer Pilze*, II. 1895.

Thus, for example, a number of oranges were inoculated by removing a small piece of the rind and sowing with *P. italicum*. After a few days the appearance of the characteristic colour showed that the fungus had taken a firm hold. The further progress of the attack, however, was at once arrested when the orange was treated with a concentrated toxic solution. The control oranges on the other hand continued rapidly to decay.

In this investigation heat has been employed for the destruction of the enzymes, but this method may not be applicable in all cases, as many toxins of this class cannot withstand such treatment. Various enzymes it is well known are unable to resist the action of strong light, and this is an agent which might be directed to the destruction of the enzymes without impairing the specific properties of the toxin. Certain chemical compounds have also been shown to possess the power of restricting or entirely inhibiting the action of various enzymes, but the special aspects of the problem must always be considered and the nature of the enzymes and toxins be taken into account.

The theory that the waste products of metabolism, after elimination of the enzymes, may be used as a means of preventing the ravages of a parasite has received strong support from the result of the experiments undertaken. How far it can be regarded as having a practical application in therapeutics is beyond the scope of the present paper, but, since the treatment may be accepted for two such widely distinct organisms as *P. destructans* and *Penicillium italicum*, it may be supposed capable of more general application and many other parasites may be checked if not effectually prevented from further progress by the adoption of this principle. The wide differences between the systems of animals and plants make comparison difficult and it is hardly possible to indicate the applicability of this work to problems in animal pathology, but it is at least suggestive and offers possibilities for a larger interpretation in the light of medical science.

My obligations are due to the Government Grant Committee of the Royal Society for the use of a Zeiss 3 mm. 1·40 apochromatic oil immersion lens.

REVIEWS

Parasitology, a Supplement to the *Journal of Hygiene.* The fundamental discoveries upon the modes of infection in such diseases as plague, malaria, sleeping sickness, piroplasmosis, yellow fever, ankylostomiasis, elephantiasis, and other important diseases, have increased our knowledge of the part played by parasites in human and animal diseases. "The successful study of such diseases, as are carried through the agency of invertebrate hosts, demands therefore not only investigations into the processes which occur in the affected vertebrate, but also observations on the structure and life-history of the pathogenic organism, and of the alternative host, or hosts, which serve to spread the disease. Thus, a knowledge of the structure and biology of mosquitoes, biting flies and ticks is necessary for a comprehensive knowledge of the etiology of malaria, trypanosomiasis, spirochaetosis and piroplasmosis, and a knowledge of fleas and their habits is essential in the study of plague. Further, recent discoveries relating to parasitic worms, especially those which produce filariasis, ankylostomiasis and various intestinal diseases, have given great stimulus to the study of entozoa."

Parasitology was founded with the view "of encouraging the study of parasitology, especially in relation to disease, by providing a means for the publication of papers relating to pathogenic and disease-transmitting parasites."

The first number (III. 1908) contains only one paper (pp. 1—100) with many illustrations by K. Jordan and N. C. Rothschild, on the anatomy and classification of those fleas which have eyes but no combs on the head and pronotum. Of these fleas one, *Loemopsylla cheopis,* is an essential factor in the spread of plague, and further research will probably show that others play an important part in the spread of certain diseases. An accurate knowledge of fleas, both in regard to their structure and biology, is therefore essential in the prevention of some diseases.

The second number (VI. 1908) contains papers on *amoebae* producing abscesses in monkeys; on the larval and pupal stages of *Anopheles maculipennis,* the mosquito largely concerned in the spread of malaria;

on the mode of multiplication of *Piroplasma bovis*, the organism which causes Texas fever or redwater in cattle, and *P. pitheci*, compared with that of *P. canis*, which produces biliary fever or malignant fever in dogs; and on the behaviour of *Spirochaetae* in bugs. This paper is of peculiar importance since many important diseases, in men, animals and birds have recently been traced to the agency of spirochaetes. Any evidence as to the mode of dissemination of these parasites is therefore of great interest. The number also includes a long paper on the structure and biology of the tick, *Haemaphysalis punctata*, which was selected for study as a common species. Such a study is at the present time specially necessary, for our knowledge of these parasites is very imperfect in spite of their great economic importance. Some of the diseases which ticks transmit, notably those due to haematozoal parasites belonging to the genus *Piroplasma*, are among the most devastating affections of domesticated animals in many parts of the world, the useful animals which suffer from piroplasmosis being cattle, sheep, goats, horses and dogs. The disease known as "Heart-water," occurring in South Africa and affecting sheep, goats and cattle, is likewise tick-transmitted. A disease of the domesticated fowl, analogous to relapsing fever in man, likewise of economic importance and occurring in different parts of the world, has also been demonstrated to be transmitted from animal to animal through the agency of ticks. The fowl disease is due to a spirochaete which is conveyed by ticks; the same holds good for human "tick-fever" and a spirochaete infection in cattle occurring in parts of Africa. Recent investigations appear to have clearly established the fact that a tick conveys spotted or Rocky Mountain fever to man. Moreover it has been claimed that a nematode worm, the *Filaria perstans*, parasitic in man, undergoes its development in a tick which is capable of conveying the parasite from one human host to another. There can be no doubt that ticks will be found, upon further investigation, to be associated in the transmission of an increasing number of diseases in animals.

The papers which have hitherto appeared, many of which are admirably illustrated, should therefore interest not only those who are especially engaged in the study of the causes and processes of human and animal diseases, but also students of agriculture, who are concerned with the welfare of domesticated animals, and the preservation of game.

Ticks, a Monograph of the Ixodoidea. Part I, Argasidae. By G. H. F. NUTTALL, C. WARBURTON, W. F. COOPER and L. E. ROBINSON. Cambridge University Press, Oct. 1908. In the foregoing review of a

paper on *Haemaphysalis punctata* in *Parasitology*, the need of a comprehensive study of ticks was pointed out. The present work supplies that need. Part I deals with the classification, structure and biology of the family, Argasidae. This part and the others which will follow it are intended to be complete in themselves, but are designed to form a volume of about 500 pages, giving a full account of the Ixodoidea or ticks. The first part is divided into two sections, the first dealing with the classification of the Argasidae which includes two genera, Argas and Ornithodorus. Numerous excellent plates and text figures illustrate all the stages of the species described, and a full list of species and keys for their identification are given.

The study of ticks has in the past been greatly hampered by the confused condition of the literature, innumerable synonyms and insufficient descriptions. The authors of this work have done a great service to future workers by abstracting the literature, collecting the synonyms and criticising and collating the descriptions of various authors.

The second section deals with the general biology of the Argasidae, the effects of their bites, and their relationship to the spread of disease.

An excellent bibliography containing the titles of about 300 papers referring to the Argasidae, printed on one side of the paper, accompanies this section. The work should be of great service to many classes of readers.